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ISOLATION, IDENTIFICATION, ANTIMICROBIAL SUSCEPTIBILITY TEST AND PUBLIC AWARENESS OF *SALMONELLA* ON RAW GOAT MEAT AT DIRE DAWA MUNICIPAL ABATTOIR, EASTERN ETHIOPIA

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A thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfilment of the requirements for the Degree of Master of Science in Veterinary Public Health

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As member of the examining board of the final MSc open defense, we certify that we have read and evaluated the Thesis entitled **"Isolation, identification, antimicrobial sensitivity test and public awareness of *Salmonella* on raw goat meat at Dire Dawa municipal abattoir, Eastern Ethiopia",** prepared by Beshatu Ferede and recommend that it be accepted as fulfilling the thesis requirement for the Degree of Master of Science in Veterinary Public health.

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LIST OF ABBREVIATIONS

AAP	American Academy of Pediatrics
AST	Antimicrobial Susceptibility Testing
BGA	Brilliant Green Agar
BPW	Buffered Peptone Water
CDC	Center for disease control and prevention
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
DDAC	Dire Dawa Administrative Council
EU	European Union
H_2S	Hydrogen Sulphide
HIV/AIDS	Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome
ISO	International Organization for Standardization
LIA	Lysine Iron agar
m.a.s.l	meters above sea level
MDR	Multi-Drug Resistant
MKTTn	Muller-Kauffmann Tetrathionet with novobiocinnin
NCCLS	National Committee for Clinical Laboratory Standards
NHS	National Health Services for Wales
NMKL	Nordic Committee on Food Analysis
OIE	Office International des Epizooties
RV	Rappaport Vassiliadis broth
Spp.	Species
Subsp.	Subspecies
TSI	Triple Sugar Iron agar
VLA	Veterinary Laboratories Agency
VP	Voges-Proskauer
WHO	World Health Organization
WTO	World Trade Organization
XLD	Xylose lysine desoxycholate
µg	Microgram

ISOLATION, IDENTIFICATION, ANTIMICROBIAL SUSCEPTIBILITY TEST AND PUBLIC AWARENESS OF *SALMONELLA* ON RAW GOAT MEAT AT DIRE DAWA MUNICIPAL ABATTIOR, EASTERN ETHIOPIA

ABSTRACT

A cross-sectional study was conducted from January 2013 to April 2014 on 249 apparently healthy slaughtered goats at municipal abattoir of Dire Dawa. Since there is no report on the status of Salmonella, the study was conducted with the objectives to isolate, identify, estimate the prevalence, to delineate the antimicrobial sensitivity and assess public awareness on Salmonella. A total of 249 goat carcass swab were systematically collected and examined for the presence of Salmonella following the standard techniques and procedures. Out of the total of 249 carcass swab samples, 44 (17.7%) were positive to Salmonella. Of all isolates, 43(97.7%) were multiple antimicrobial resistant and highest level of resistance was observed for tetracycline (100%), nitrofurans (100%), streptomycin (81.8%) and kanamycin (79.5%). However, all isolates were susceptible to ciprofloxacin. The knowledge, attitude and practices of goat meat handlers and consumers and provided to the consumers were found poor. Therefore, goat meat provided to the city was found less hygienic and not safe for human consumption. Thus, urgent intervention program is essential to minimize the risk associated with consumption of goat meat contaminated with Salmonella. Finally, the authors recommended that the use of standardized procedures in slaughtering and handling of goat meat, provision of training on best practice of handling of meat for handlers and raising the level of awareness of people.

***Key words**: Abattoir, Antimicrobial resistance, butcher, goat meat, hygiene, identification, isolation, Salmonella*

1. INTRODUCTION

Food safety remains a critical issue with outbreaks of foodborne illness resulting in substantial costs to individuals, the food industry and the economy (Kaferstein *et al*., 1997). Despite advances in food science and technology, foodborne diseases remain one of the major public health and economic problems all over the world (WHO, 1995 and Legnani *et al*., 2004). The risk of foodborne illness has increased markedly over the last 20 years, with nearly a quarter of the population at higher risk for illness (CDC, 2003; 2004). For instance in the United States, 76 million people get sick, 325,000 hospitalizations, 5,000 Americans die each year from foodborne illness and 2,366,000 cases, 21,138 hospitalizations and 718 deaths in England and Wales (Mead *et al.*,1999 and Adak *et al.*, 2002). There are about 5.4 million cases of foodborne disease in Australia each year (OzFoodNet, 2006). Hence, trends in foodborne illness in the developed countries indicate that the incidence of foodborne illness is increasing, and that it is likely to remain a threat to public health well into this century (Crerar *et al*., 1996).

There are many and varied sources of organisms causing food poisoning. Most cases of food poisoning are caused by bacteria which arise from animal, human or environmental sources (Gracey *et al*., 1999). Contaminated raw meat is one of the main sources of foodborne illnesses (Bhandare *et al*., 2007). Specific sources that contribute microbial contamination to animal carcasses and to fresh meat during slaughter and dressing include the faeces, the hide, water, air, intestinal contents, lymph nodes, processing equipment, and humans (Sofos, 2005), and can be transferred to the carcass during skin removal and evisceration (Hansson *et al*., 2000; Reid *et al*., 2002). The types of microorganisms and extent of contamination present on the final product are influenced by sanitation procedures, hygienic practices, application of food safety interventions, type and extent of product handling and processing, and the conditions of storage and distribution (Sofos, 2005). There are four major pathogens that have frequently been associated with meat and meat products including *Salmonella* species, *Campylobacter* species, *Listeria monocytogenes*, and *Escherichia coli* O157:H7. These organisms have been linked to a number of cases of human illness (Mershal *et al*., 2010).

Salmonella is the most frequently reported cause of foodborne illness (Birhaneselassie and Williams, 2013). Foodborne salmonellosis often follows consumption of contaminated animal products, which usually results from infected animals used in food production or from contamination of the carcasses or edible organs (Alemayehu *et al*., 2002). *Salmonella* infection in meat animals arises from intensive rearing practices and the use of contaminated feeds (Ejeta *et al*., 2004). Cross-contamination of carcasses with *Salmonella* can also occur during slaughtering operations (Baird-Parker, 1990). Stress associated with transport of animals to abattoir augments shedding of *Salmonella* by carrier animals and this may contribute to the spread of the organism to other animals in the slaughter plant (Isaacson *et al*., 1999).

Slaughtering procedures potentially involve many risks of both direct and cross contamination of carcasses and meat surfaces. During slaughter, faecal contamination of edible organs with subsequent contamination of the carcass may occur. This can be carried through all slaughter procedures up to the processing of the raw products, which are important sources of *Salmonella* in the human food chain (Edwards *et al*., 1997). Contamination of equipment, utensils and hands of workers can spread *Salmonella* to uncontaminated carcasses and parts, which can occur in subsequent handling, processing, transport, storage, distribution and preparation for consumption (Ejeta *et al*., 2004).

Salmonellosis causes significant morbidity and mortality in both humans and animals and has a substantial global socioeconomic impact (Tassios *et al*., 1997). There are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths worldwide due to *Salmonella* (Bhunia, 2008). Mortality due to *Salmonella* infections is mainly a health problem in developing countries, but morbidity due to acute *Salmonella* infections also has important socio-economic impact in industrialised nations (Hansen-Wester and Hensel, 2001). *Salmonella* infections in the United States account for roughly 19,336 hospitalizations, 17,000 quality adjusted life years lost and $3.3 billion in total medical expenditures and lost productivity each year (Batz *et al*., 2011). For human salmonellosis in the Netherlands, the costs are estimated to be between 32 and 90 million Euro per year (van Pelt and Valkenburgh, 2001).

Salmonella infections are very common and an important public health problem in many parts of the world. Studies in different countries indicated that *Salmonella*e are wide spread in small ruminants (Nabbut and Al-Nakhli, 1982 and Chandra *et al*., 2007). Research to date, as well as unpublished reports from different health institutions in Ethiopia have indicated that salmonellosis is a common problem and also showed the presence of a number of serogroups/ serotypes in humans, animals, animal food products and other foods (Nyeleti *et al*., 2000; Muleta and Ashenafi, 2001; Molla *et al*., 2003; Tibaijuka *et al*., 2003; Woldemariam *et al*., 2005, Asrat, 2008 and Akafete and Haileleul, 2011).

Moreover, an increase in the resistance of *Salmonella* to commonly used antimicrobials has been also noted in both public health and veterinary sectors in Ethiopia (Molla *et al.,* 1999; Molla *et al.,* 2003 and Asrat, 2008). The extensive use of the first line drugs has led to the development of multiple drug resistance at a level which could pose a serious problem in the near future (Getenet, 2008). Although, little study has so far been undertaken to isolate *Salmonella* from goat's meat in Ethiopia (Molla *et al.,* 1999, Woldemariam *et al*., 2005; Wassie, 2004 and Akafete and Haileleul, 2011) from central part of the country and export abattoirs, there was no report regarding the status of the *Salmonella* from Dire Dawa municipal abattoir.

Thus, the objectives of the study were:-

- To isolate and identify *Salmonella* from goat meat slaughtered at Dire Dawa municipal abattoir
- To estimate the prevalence of *Salmonella* from goat meat slaughtered at Dire Dawa municipal abattoir
- To delineate the antimicrobial sensitivity of the isolated pathogen
- To assess the knowledge, attitude and practice of abattoir workers, butchers and consumers on meat hygiene and food safety

2. LITERATURE REVIEW

2.1. Overview of food hygiene and food safety

Foodborne diseases remain a real and formidable problem in both developed and developing countries, causing great human suffering and significant economic losses. Up to one third of the population of developed countries may be affected by foodborne diseases each year, and the problem is likely to be even more widespread in developing countries, where food and water-borne diarrhoeal diseases kill an estimated 2.2 million people each year, most of them Children (FAO/WHO, 2006). The problem is severe in developing countries due to difficulties in securing optimal hygienic food handling practices. In developing countries, up to an estimated 70% of cases of diarrheal disease are associated with the consumption of contaminated food (Knife and Abera, 2007).

Food safety is therefore a fundamental public health concern, and achieving a safe food supply poses major challenges for national food safety officials. Changing global patterns of food production, international trade, technology, public expectations for health protection and many other factors have created an increasingly demanding environment in which food safety systems operate. An array of foodborne hazards both familiar and new, pose risks to health and obstacles to international trade in foods. These risks must be assessed and managed to meet growing and increasingly complex sets of national objectives (CAC, 2007).

2.1.1. Food hygiene and food safety practices in Ethiopia

Foodborne diseases are common in developing countries including Ethiopia because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment, and lack of education for food handlers (WHO, 2004). National Hygiene and Sanitation Strategy program (MoH, 2005) reported that in Ethiopia more than 250,000 children die every year from sanitation and hygiene related diseases and about 60% of the disease burden was related to poor hygiene and sanitation in Ethiopia. Unsafe sources, contaminated raw food items, improper food storage, poor personal hygiene during food preparation, inadequate cooling and reheating of food items and a prolonged time lapse between preparing and consuming food items were mentioned as contributing factors for outbreak of foodborne diseases (Linda du and Irma, 2005).

Studies conducted in different parts of the country showed the poor sanitary conditions of catering establishments and presence of pathogenic organisms like *campylobacter*, *Salmonella, Staphylococcus aureus, Bacillus cereus and Escherichia coli,* (Bayleyegn *et al*., 2003; Abera *et al*., 2006; Knife and Abera, 2007; Tefera *et al*., 2009 and Mekonnen *et al*., 2013).

Of the foods intended for humans, those of animal origin tend to be most hazardous unless the principles of food hygiene are employed. Animal products such as meats, fish and their products are generally regarded as high-risk commodity in respect of pathogen contents, natural toxins and other possible contaminants and adulterants (Yousuf *et al*., 2008). Bacterial contamination of meat products is an unavoidable consequence of meat processing (Jones *et al*., 2008). Even if data regarding meat borne diseases in Ethiopia are extremely scarce, a few studies conducted in different parts of the country have shown the public health importance of several bacterial pathogens associated with foods of animal origin (Bayleyegn *et al*., 2003; Ejeta *et al*., 2004: Adem *et al*., 2008; Kumar *et al*., 2009 and Tefera *et al*., 2009). *Salmonella* remains a leading etiological agent in bacterial foodborne diseases (D'Aoust, 1991).

2.2. Overview of *Salmonella*

2.2.1. Historical background

The *Salmonella* bacterium was first described by Theobald Smith (1859-1934) and then in 1885, two American veterinarians, Salmon and Smith isolated the bacterium causing hog cholera from infected pigs (Salmon and Smith, 1886). The name *Salmonella* was subsequently adopted in honor of Dr. Salmon. Over the decades following the pioneering work of Salmon and Smith, many other *Salmonella* were isolated from both animals and humans (Widal, 1896; Getenet, 2008). The antigenic classification or serotyping of *Salmonella* used today is a result of years of study of antibody interactions with bacterial surface antigens by Kauffman and White in the 1920s to 1940s (Kauffmann, 1950). According to this Kauffmann-White scheme, each *Salmonella* serotype is recognized by its possession of a particular lipopolysaccharide (LPS) or O antigen and a flagellar or H antigen. This led to the description of more than 2500 serotypes at present (Brenner *et al*., 2000; Popoff *et al*., 1998 and Popoff *et al*., 2004).

2.2.2. Classification and nomenclature

Historically *Salmonella* had been named based on the original places of isolation such as *Salmonella* London and *Salmonella* Indiana. This nomenclature system was replaced by the classification based on the susceptibility of isolates to different selected bacteriophages which is also known as phage typing. Phage typing is generally employed when the origin and characteristic of an outbreak must be determined by differentiating the isolates of the same serotype. It is very reproducible when international standard sets of typing phages are used More than 200 definitive phage types (DT) have been reported so far. For example, *S.* Typhimurium DT104 designates a particular phage type for Typhimurium isolates (Hanes, 2003; Andrews and Baumler, 2005 and Pui *et al*., 2011).

Epidemiologic classification of *Salmonella* is based on the host preferences. The first group includes host-restricted serotypes that infect only humans such as *S.* Typhi. The second group includes host-adapted serotypes which are associated with one host species but can cause disease in other hosts serotypes such as *S.* Pullorum in avian. The third group includes the remaining serotypes. Typically, *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Heidelberg are the three most frequent serotypes recovered from humans each year (Gray and Fedorka-Cray, 2002 and Boyen *et al*., 2008).

The genus consists of two species: the first is *S. enterica* which is divided into six subspecies(Figure 1): *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae* and *S. enterica* subsp. *indica*; and the second is *S. bongori* (formerly called *S. enterica* subsp. *bongori*) (WHO, 2003c). *Salmonella enterica* subspecies I is mainly isolated from warm-blooded animals and accounts for more than 99% of clinical isolates whereas remaining subspecies and *S. bongori* are mainly isolated from cold-blooded animals and account for less than 1% of clinical isolates. As an example, the Kauffmann species *Salmonella T*yphimurium is now designated as *Salmonella enterica* subspecies I serotype Typhimurium. Under the modern nomenclature system, the subspecies information is often omitted and culture is called *S. enterica* serotype Typhimurium and in subsequent appearance, it is written as *S.* Typhimurium. This system of nomenclature is used nowadays to bring uniformity in reporting (Andrews and Baumler, 2005 and Parry, 2006).

Kauffmann-White scheme classifies *Salmonella* according to three major antigenic determinants composed of flagellar H antigens, somatic O antigens and virulence (Vi) capsular K antigens. This was adopted by the International Association of Microbiologists in 1934. Agglutination by antibodies specific for the various O antigens is employed to group *Salmonella*e into the 6 serogroups: A, B, C1, C2, D and E. For instance, *S.* Paratyphi A, B, C and *S.* Typhi express O antigens of serogroups A, B, C1 and D, respectively. More than 99% of *Salmonella* strains causing human infections belong to *Salmonella enterica* subspecies *enterica*. Although not common, cross-reactivity between O antigens of *Salmonella* and other genera of *Enterobacteriaceae* do occur (Pui *et al*., 2011).

Therefore, further classification of serotypes is based on the antigenicity of the flagellar H antigens which are highly specific for *Salmonella* (Scherer and Miller, 2001). In brief, O antigens are lipopolysaccharide (LPS) of the outer bacterial membrane. They are heat stable, resistant to alcohol and dilute acids. H antigens are heat-labile proteins associated with the peritrichous flagella and can be expressed in one of two phases. The phase 1 H antigens are specific and associated with the immunological identity of the particular serovars whereas phase 2 antigens are non-specific antigens containing different antigenic subunit proteins which can be shared by many serovars. K antigens which are heat-sensitive carbohydrates are produced by *Salmonella* serovars that express a surface-bound polysaccharide capsular antigen (Hu and Kopecko, 2003; Yousef and Carlstrom, 2003).

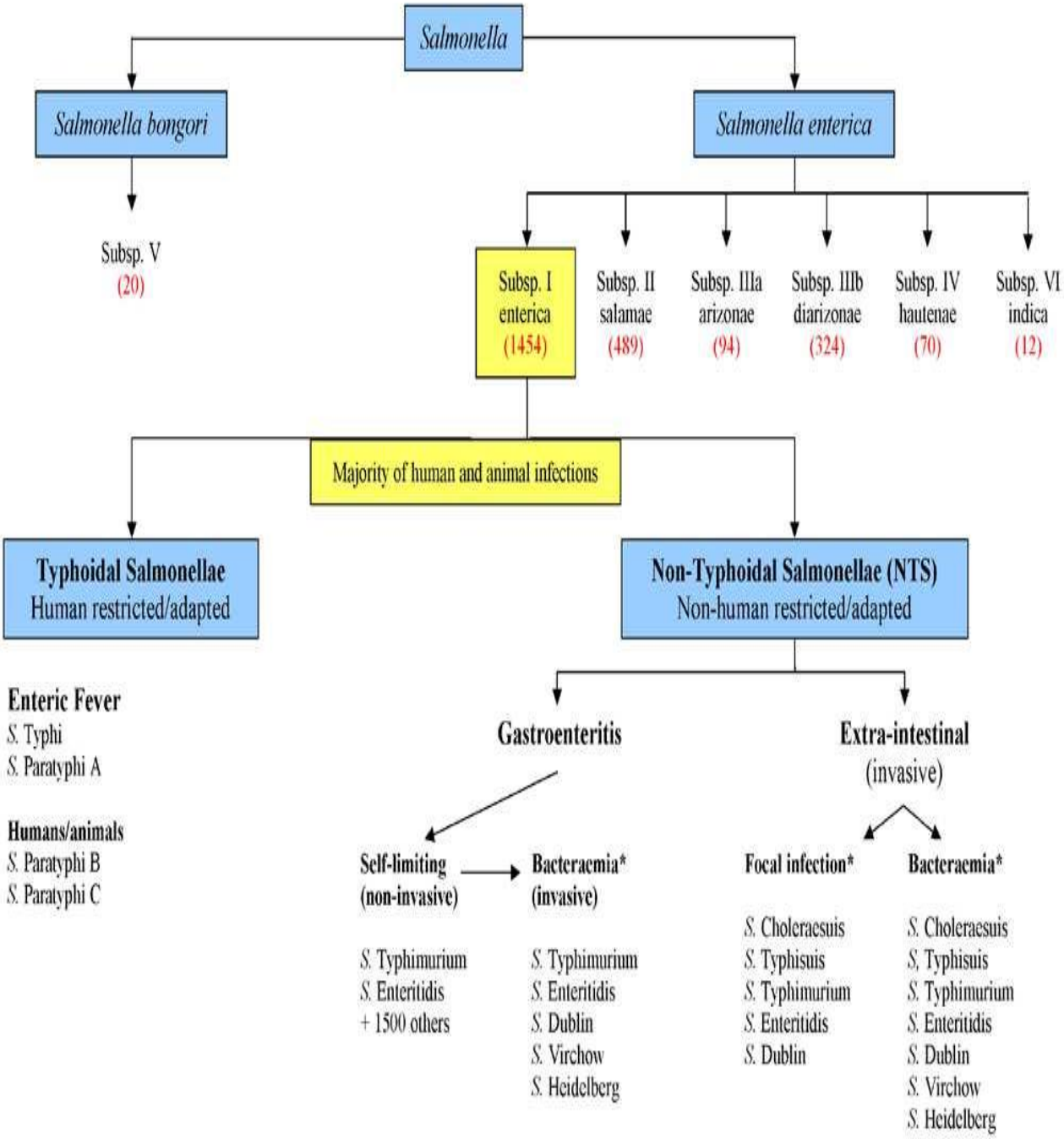

Figure 1: Classification of the genus *Salmonella*

Source: Langridge *et al*., (2008).

Note: Numbers in brackets indicate the total number of serotypes included in each subspecies.

* Common serotypes are listed but other serotypes may cause bacteraemia or focal infection; subsp = subspecies.

2.2.3. General characteristics of Salmonella

Salmonella make up a large genus of gram-negative bacilli within the family *Enterobacteriaceae* and it constitute a genus of more than 2300 serotypes that are highly adapted for growth in both humans and animals and that cause a wide spectrum of disease. The growth of *S. typhi* and *S. paratyphi* is restricted to human hosts, in whom these organisms cause enteric (typhoid) fever. The remainder of *Salmonella* serotypes, referred to as non-typhoidal *Salmonella*, can colonize the gastrointestinal tracts of a broad range of animals, including mammals, reptiles, birds, and insects. More than 200 of these serotypes are pathogenic to humans, in whom they often cause gastroenteritis and can also be associated with localized infections and/or bacteremia (Fuaci and Jameson, 2005).

Members of the genus *Salmonella* are ubiquitous pathogens found in humans and livestock, wild animals, reptiles, birds, insects (Getenet, 2008) and can multiply under various environmental conditions outside the living hosts (Pui *et al*., 2011). *Salmonella*e are gram-negative, non-spore forming, facultative anaerobic bacilli, and 2 to 3 by 0.4 to 0.6 µm in size (Getenet, 2008). They do not require sodium chloride for growth, but can grow in the presence of 0.4 to 4%. Most *Salmonella* serotypes grow at temperature range of 5 to 47°C with optimum temperature of 35 to 37°C but some can grow at temperature as low as 2 to 4°C or as high as 54°C . They are sensitive to heat and often killed at temperature of 70°C or above. *Salmonella* grow in a pH range of 4 to 9 with the optimum between 6.5 and 7.5. They require high water activity (aw) between 0.99 and 0.94 (pure water aw=1.0) yet can survive at water activity less than 0.2 such as in dried foods. Complete inhibition of growth occurs at temperatures less than 7°C, pH less than 3.8 or water activity less than 0.94 (Pui *et al*., 2011).

Like other members of the family *Enterobacteriaceae*, they produce acid on glucose fermentation; reduce nitrates to nitrite, and don't produce cytochrome oxidase. In addition; all *Salmonella*e except *S. gallinarum-pullorum* are motile by means of peritrichous flagella, and all but *S. typhi* produce gas (H_2S) on sugar fermentation (Fuaci and Jameson, 2005 and Getenet, 2008). *Salmonella* are non- capsulated except *S.* Typhi, *S.* Paratyphi C and some strain of *S*. Dublin (Getenet, 2008).

2.2.4. Geographic distribution and host range

Salmonella is one of the leading causes of bacterial foodborne disease in industrialized as well as developing countries even though the incidence seems to vary between countries (Radostits *et al*., 1994; D'Aoust, 1997; Molla *et al*., 2003 and Chiu *et al*., 2004). The wide variations in the national prevalence of Salmonellosis likely arise from limited scope of studies and lack of coordinated epidemiological surveillance systems, under-reporting of cases and the presence of other diseases considered being of high priority (Radostits *et al*., 1994 and Molla *et al*., 2003).

The epidemiology of salmonellosis is complex largely because there are more than 2,500 distinct serotypes (serovars) with different reservoirs and diverse geographic incidences. Changes in food consumption, production, and distribution have led to an increasing frequency of multistate outbreaks associated with fresh produced and processed foods (Rounds *et al*., 2010).

According to the WHO Global Salm-Surv, during 2000-2002, *S.* Enteritidis was by far the most common serotype reported from humans globally. In 2002, it accounted for 65% of all isolates, followed by *S.* Typhimurium at (12%) and *S.* Newport at (4%). Among non-human isolates, S. Typhimurium was the most commonly reported serotype in all the three years, accounting for (17%) of isolates in 2002 followed by *S.* Heidelberg (11%) and S. Enteritidis (9%). *Salmonella* Enteritidis, *S.* Typhimurium and *S.* Typhi were ranked among the fifteen most common human serotypes in all regions of the world throughout the three year study period. *Salmonella* Agona, *S.* Infantis, *S.* Montevideo, *S.* Saintpaul, *S.* Hadar, *S.* Mbandaka, *S.* Newport, *S.* Thompson, *S.* Heidelberg and *S.* Virchow were also widespread. In Africa in 2002, *S.* Enteritidis and *S.* Typhimurium were each reported from approximately one fourth of isolates from humans (Galanis *et al*., 2006 and Swaminathan *et al*., 2006).

2.2.5. Reservoir host and source of infection

Salmonellosis is the most common foodborne disease in both developing and developed countries, although incidence rates vary according to the country (Stevens *et al*., 2006). The fecal wastes from infected animals and humans are important sources of bacterial contamination of the environment and the food chain (Ponce *et al*., 2008). Members of *Salmonella enterica* subspecies *enterica* are widely distributed in the environment and in the intestinal tracts of animals (Anjum *et al*., 2011). People can become infected following a failure of personal hygiene after contact with infected animals and or other infected people. Environmental contamination, especially untreated water is also important (Gracey *et al*., 1999). Most human infections are acquired through consumption of contaminated food of animal origin (Gracey *et al*., 1999 and Anjum *et al*., 2011).

Foods of animal origin, particularly meat, poultry, and, in some instances, unpasteurized egg products are considered to be the primary sources of human salmonellosis (Tauxe, 1991; Nielsen *et al.*, 1995; Wray and Davies, 2000; Acha and Szyfres, 2001; White *et al.*, 2001). It has been reported that livestock and their products can contribute to as much as 96% of the total *Salmonella* infection in humans (Dahal, 2007). Most of these food products, e.g. beef, mutton and poultry, become contaminated during slaughter and processing, from the gut contents of healthy excreting animals. In the same way, all food that is produced or processed in a contaminated environment may become contaminated with *Salmonella*e and be responsible for outbreaks or separate cases of disease as a result of faults in transport, storage, or preparation (D'Aoust, 1997). Unlike *S. typhi* and *S. paratyphi*, whose only reservoir is humans; non-typhoidal salmonellosis is acquired from multiple animal reservoirs (Fuaci and Jameson, 2005).

A less common source of non-typhoidal *Salmonella* infections is exposure to pets, especially reptiles. Fecal carriage rates in reptiles can be more than 90%. It is estimated that approximately 74,000 infections with *Salmonella* result from exposure to reptiles and amphibians in the United States each year (AAP, 2013). Iince 1986, an increase in the popularity of non-banned reptiles, including iguanas, has been followed by increases in rates of *Salmonella* infections. Other pets, including African hedgehogs, snakes, birds, rodents, baby chicks, ducklings, dogs, and cats, can also serve as potential vectors (Fuaci and Jameson, 2005).

2.2.6. Mode of transmission

Salmonella infection appears to be one of the most common examples of an enteric disease that is transmitted from animals to humans. The transmission occurs both through food products, such as meat, dairy products, and eggs, and by direct contact between animals and humans through the fecal-oral route (Olsvik, *et al*., 1985).

Foodborne salmonellosis often follows consumption of contaminated animal products such as raw meat, poultry and eggs. Not washing fresh fruits and vegetables before eating them, as well as not thoroughly cleaning work surfaces used to prepare raw meat and other foods in the kitchen can also be source of *Salmonella*. Food can also be contaminated by food handlers who do not thoroughly wash their hands with soap after handling raw meat or after using the bathroom (WHO, 1989). *Salmonella* infections are primarily of foodborne origin but can also occur through contact with infected animals, humans, other feces (Rounds *et al*., 2010).

The main mode of transmission is from food products contaminated with animal products or waste most commonly eggs and poultry but also undercooked meat, unpasteurized dairy products, seafood, and fresh produced. *S. enteritidis* associated with chicken eggs is emerging as a major cause of foodborne disease. Approximately 1 in 20,000 eggs is thought to be infected with *S. enteritidis*. Between 1974 and 1994, there was a fivefold increase (from 5% to 25%) in the isolation of *S. enteritidis* from eggs in the United States; in 1998, the U.S. Department of Agriculture estimated that 80% of all salmonellosis cases were caused by infected eggs (Fuaci and Jameson, 2005).

2.2.7. Carrier states and susceptibility

Stool cultures remain positive for four to five weeks after infection. Morbidity and mortality associated with salmonellosis are highest among the elderly, infants, and immune compromised individuals, including those with hemoglobinopathies and those infected with HIV or with pathogens that cause blockade of the reticuloendothelial system (e.g., patients with bartonellosis, malaria, schistosomiasis, or histoplasmosis) (Fuaci and Jameson, 2005).

Conditions that decrease stomach acidity like an age of less than one year, antacid ingestion, or achlorhydric disease or conditions that decrease intestinal integrity (inflammatory bowel disease, history of gastrointestinal surgery, or alteration of the intestinal flora by antibiotic administration) increase susceptibility to *Salmonella* infection (Fuaci and Jameson, 2005).

2.2.8. Virulence factors

The outcome of a *Salmonella* infection is determined by the status of the host and status of the bacterium. The status of the bacterium is determined by the so called virulence factors which is described as follows (Van Asten and van Dijk, 2005).

***Salmonella* Pathogenisity Islands (SPIs) -**The majority of virulence genes of *Salmonella* are clustered in regions distributed over the chromosome called *Salmonella* pathogenicity islands (McClelland *et al*., 2001). The SPIs are of major importance for the virulence of *S. enterica*. Hallmarks of *Salmonella* virulence, such as cell invasion, intracellular survival and the production of Vi antigens capsule are encoded by SPIs. Until recently more than 10 SPIs have been identified on the *Salmonella* chromosome, but SPI-1 and SPI-2 is the central for pathogenesis of *Salmonella* infections (Hansen-Wester and Hensel, 2001).

All types of *S. enterica* have two large clusters of genes known as *Salmonella* Pathogenicity Island one and two. *Salmonella* Pathogenicity Island one encodes genes necessary for invasion of intestinal epithelial cells and induction of intestinal secretory and inflammatory response (Galyov *et al*., 1997). *Salmonella* lacking a functional SPI-1 Type three secretion system are unable to invade epithelia cells and induce cytokine synthesis (Hobbie *et al*., 1997).

Salmonella Pathogenicity Island 2 encodes genes essential for intracellular replication and necessary for establishment of systemic infection beyond the intestinal epithelium (Hensel, 2006). The function of the SPI-2 encoded Type III secretion system is required to protect the pathogens within the *Salmonella* containing vacuole (SCV) against the effectors functions of innate immunity. It has been reported that SPI-2 prevents localization of the phagocyte oxidase (Vazquez-Torres *et al*., 2000) and the inducible nitric oxide synthases to the SCV (Chakravortty *et al*., 2002).

Type III secretion systems- Central to the pathogenesis of *S. enterica* is the function of specialized protein secretion systems, known as Type III secretion system (TTSS). TTSS are specialized virulence devices that have evolved indirect translocation of bacterial virulence proteins into the host cell cytoplasm. Type III secretion systems are composed of several proteins that form a remarkable needle-like organelle in the bacterial envelope (Galan, 1998). So far the presence of two SPIs (SPI-1 and SPI-2) each encoding a TTSS, have been described for *Salmonella* species and may reflect the flexibility of this highly successful pathogen in causing different forms of diseases (Fierer and Guiney, 2001).

Regulatory proteins, toxins, plasmids and Vi antigens- Regulatory proteins that control the synthesis of multiple proteins at the level of gene transcription are also essential to *Salmonella* pathogenesis (Behlau and Miller, 1993).

Non-typhoidal *Salmonella* also carry a variety of virulence plasmids which might play a role in multiplication inside the cell, destabilizing the cytoskeleton of the eukaryotic cell and also might be involved in resistance of *Salmonella* species to the bacteriolytic activity of serum. Enterotoxin may also play a role in *Salmonella* gastroenteritis. An enterotoxin antigenically similar to Cholera toxin also has been identified (Aguero *et al*., 1991). Flagella phase variation that is exploited by the majority of flagellated *Salmonella* might be related to escaping the host defense system (Van Asten and van Dijk, 2005). The Vi antigen of *S.* Typhi prevents antibody mediated opsonization, increases resistance to peroxide, and confers resistance to complement activation by the alternative pathway and to complement mediated lysine (Looney and Steigbigel, 1986).

2.2.9. Pathogenesis

Salmonellosis in the human host is generally associated with *Salmonella enterica* subspecies *enterica* and acute infections can present in one of four ways: enteric fever, gastro-enteritis, bacteremia, and extra intestinal (EI) focal infection. As with other infectious diseases the course and outcome of the infection are dependent upon a variety of factors including inoculating dose, immune status of the host and genetic background of both host and infecting organism (Getenet, 2008). Broadly speaking the *Salmonella enterica* from human infections can be subdivided in to two groups: the enteric fever (typhoidal) group and non-typhoidal *Salmonella* (NTS), which typically cause gastroenteritis but can cause invasive disease under certain conditions (Selander *et al*., 1990).

All *Salmonella* infections begin with the ingestion of organisms in contaminated food or water (Francis *et al*., 1992 and Fuaci and Jameson, 2005). The infectious dose of *Salmonella* varies from 103 to 106 colony-forming units. This variability probably reflects the ability of *Salmonella*e to resist the low pH of the stomach a powerful component of host defense (Fuaci and Jameson, 2005). After leaving the stomach, *Salmonella* must traverse the mucosal layer overlaying the epithelium of the small intestine. After crossing the mucosal layer overlaying the intestinal epithelium, *Salmonella* interacts with both enterocytes and Microfolds cells (Mcells) (Francis *et al*., 1992). The organisms are rapidly internalized and transported into submucosal lymphoid tissue where they may enter into systemic circulation. *Salmonella* have also the ability to induce non phagocytic epithelial cells by a process known as bacterial mediated endocytosis. This process involves the formation of large membrane ruffles around the organism and cytoskeleton rearrangement (Francis *et al*., 1992). *Salmonella* is then internalized within bound vacuoles through which organisms' trancytose from the apical to the basolateral surface (Rathman *et al*., 1997). Once it crosses the intestinal epithelium, *Salmonella* serotypes that cause systemic infections entermacrophages, and migration of infected macrophages to other organs of reticulo-endothelial systems probably facilitates the dissemination of bacteria in the host (Getenet, 2008).

Gastroenteritis due to NTS may persist with fever, nausea, vomiting, abdominal pain and symptoms may continue for over a week. In contrast, the early symptoms of enteric fever are often vague, and may include a dry cough, severe headache, anorexia, fever and a tendency to constipation rather than diarrhoea (Parry *et al*., 2002). If enteric fever is not treated on time, serious complication like hemorrhage from ulcers can occur during the third week of illness or perforation of the peyer's patches (PP) can cause generalized peritonitis and septicemias; these are the commonest cause of death in typhoid fever. With the introduction of early and appropriate antibiotic therapy, the average case fatality rates for typhoid are less than 1% (Everest *et al*., 2001).

2.2.10. Clinical futures

Both human and animals are susceptible to *Salmonella* infection. While some of this infections couses disease, the majority probably leads to subclinical cases resulting in a healthy carier state with intermitent excreation of the *Salmonella* in faeces . Whether a human develop disease folowing ingestion of *Salmonella* depend on dose of organism, the speciese of *Salmonella* and up on the specific and non-specific immunological factors. Speciese such as *S. typhimurium* and *S. enteritidis* usualy causes gastrointeritis (food poisoning). The majority of food poisoning outbreks coused by *Salmonella* follow the consuption of food directily or indirecly asssociated with infection in animals. The chaine of transmission is often from contaminated animal food staffs to animal and then from contaminated animal carcasses to man (Quinn *et al*., 1999).

Salmonella infections in animals

Salmonella have a wide variety of domestic and wild animal hosts. The infection may or may not be clinically apparent. In the subclinical form, the animal may have a latent infection and harbor the pathogen in its lymph nodes, or it may be a carrier and eliminate the agent in its fecal material briefly, intermittently, or persistently. In domestic animals, there are several well-known clinical enteritis due to species-adapted serotypes, such as *S.* pullorum or *S.* abortus equi. Other clinically apparent or in apparent infections are caused by serotypes with multiple hosts (PAHO, 2001).

The principal causes of clinical salmonellosis in cattle are serotype Dublin and *S.* Typhimurium. Other serotypes can sometimes be isolated from sick animals. Salmonellosis in adult cattle occurs sporadically, but in calves it usually acquires epizootic proportions. The disease generally occurs when stress factors are involved. Serotype *dublin*, adapted to cattle, has a focal geographic distribution. In the Americas, outbreaks have been confirmed in the western United States, Venezuela, Brazil, and Argentina. It also occurs in Europe and South Africa. In adult cattle, the disease begins with high fever and the appearance of blood clots in the feces, followed by profuse diarrhea, and then a drop in body temperature to normal. Signs of abdominal pain are very pronounced. The disease may be fatal within a few days or the animal may recover, in which case it often becomes a carrier and new cases appear. Calves are more susceptible than adults, and in them the infection gives rise

to true epidemic outbreaks, often with high mortality. Septicemia and death are frequent in newborns. The carrier state is less frequent among young animals and occurs primarily in adult cattle. The infection is almost always spread by the feces of a cow that is shedding the agent, but it may also originate from milk (PAHO, 2001).

Swine are host to numerous *Salmonella* serotypes and are the principal reservoir of *S.* choleraesuis. Serotypes that attack swine include *S.* enteritidis, *S.* Typhimurium, and *S.* dublin. *S. choleraesuis* is very invasive and causes septicemia; it may be isolated from the blood or from any organ. Swine are particularly susceptible and experience epidemic outbreaks between 2 and 4 months of age, but the infection also appears in mature animals, almost always as isolated cases. The most frequent symptoms are fever and diarrhea. The infection usually originates from a carrier pig or contaminated food. Infection by other serotypes may sometimes give rise to serious outbreaks of salmonellosis with high mortality. Because of the frequency with which swine are infected with different types of *Salmonella*e, pork products have often been a source of human infection (PAHO, 2001).

Cases of clinical salmonellosis in sheep and goats are infrequent. The most common serotype found in gastroenteritis cases is *S. typhimurium*, but many other serotypes have also been isolated. Serotype *S. abortus ovis*, which causes abortions in the last two months of pregnancy and gastroenteritis in sheep and goats, seems to be restricted to Europe and the Middle East (PAHO, 2001). Horses are also susceptible to *Salmonella*e, particularly *S. typhimurium*. *Salmonella* enteritis occurs in these animals, sometimes causing high mortality. Calves suffer from acute enteritis with diarrhea and fever; dehydration may be rapid. Nosocomial transmission has been seen in hospitalized horses (Bauerfeind *et al.*, 1992).

In recent years, a high prevalence of infection caused by numerous serotypes has been confirmed in cats and dogs. These animals may be asymptomatic carriers or may suffer from gastroenteritic salmonellosis with varying degrees of severity. Dogs can contract the infection by eating the feces of other dogs, other domestic or peridomestic animals, or man. Dogs and cats can also be infected by contaminated food. In addition, dogs can transmit the disease to man. Treatment for these animals consists mainly of fluid and electrolyte replacement (PAHO, 2001).

Two serotypes, *S. pullorum* and *S. gallinarum*, are adapted to domestic fowl. They are not very pathogenic for man, although cases of salmonellosis caused by these serotypes have been described in children. Many other serotypes are frequently isolated from domestic poultry; for that reason, these animals are considered one of the principal reservoirs of *Salmonella*e. Pullorum disease, caused by serotype *S. pullorum*, and fowl typhoid, caused by *S. gallinarum*, produce serious economic losses on poultry farms if not adequately controlled. Both diseases are distributed worldwide and give rise to outbreaks with high morbidity and mortality. Pullorum disease appears during the first 2 weeks of life and causes high mortality. The agent is transmitted vertically as well as horizontally. Carrier birds lay infected eggs that contaminate incubators and hatcheries. Fowl typhoid occurs mainly in adult birds and is transmitted by the fecal matter of carrier fowl. On an affected poultry farm, recuperating birds and apparently healthy birds are reservoirs of infection. *Salmonella* un-adapted to fowl also infect them frequently. Nearly all the serotypes that attack man infect fowl as well. Some of these serotypes are isolated from healthy birds. The infection in adult birds is generally asymptomatic, but during the first few weeks of life, its clinical picture is similar to pullorum disease (loss of appetite, nervous symptoms, and blockage of the cloaca with diarrheal fecal matter). The highest mortality occurs during the first two weeks of life. Most losses occur between six and ten days after hatching (PAHO, 2001).

Rodents become infected with the serotypes prevalent in the environment in which they live. Rodents found in and around food processing plants can be an important source of human infection. Of 974 free-living wild animals examined in Panama, 3.4% were found to be infected, principally by serotype *S.* enteritidis and, less frequently, by *S.* arizonae (*Arizona hinshawii*) and *Edwardsiella*. The highest rate of infection (11.8%) was found among the 195 marsupials examined. Outbreaks of salmonellosis among wild animals held in captivity in zoos or on pelt farms are not unusual. *Salmonella* infection in cold-blooded animals has merited special attention. An infection rate of 37% was found in 311 reptiles examined live or necropsied at the National Zoo in Washington, D.C. The highest rate of infection was observed in snakes (55%) and the lowest in turtles (3%). The *Salmonella*e isolated were 24 different serotypes formerly classified under the common name of *S. enteritidis*, 1 strain of *S. choleraesuis*, and 39 of *S. arizonae*. No disease in their hosts was attributed to these bacteria, but they may act together with other agents to cause opportunistic infections (PAHO, 2001).

Salmonella infections in humans

Salmonella infections in humans can range from a self-limited gastroenteritis usually associated with non-typhoidal *Salmonella* (NTS) to typhoidal fever with complications such as a fatal intestinal perforation (OIÉ, 2000). Non-typhoidal *Salmonella* is one of the principal causes of food poisoning worldwide with an estimated annual incidence of 1.3 billion cases and 3 million deaths each year (Torpdahl *et al*., 2007). Outbreaks of salmonellosis have been reported for decades, but within the past 25 years the disease has increased in incidence in many continents. The disease appears to be most prevalent in areas of intensive animal husbandry (OIÉ, 2000).

The incubation period in people is variable but is usually between 12 and 36 hours. The typical presenting symptom is diarrhea but this may be accompanied by nausea and abdominal pain, although vomiting is not usual. There may also be a headache and fever. While the infection is normally self-limiting and does not require antibiotic treatment, occasionally, with more invasive *Salmonella* such as *S. Virchow*, bacteremia can occur. The infection is rarely fatal in people (Gracey *et al*., 1999).

Salmonellosis is most commonly caused by *S. enterica* subsp. *typhimurium* or *S. enterica* subsp. *enteritidis*. Secondly, *S. enterica* subsp. *typhi* and *S. enterica* subsp. *paratyphi* are the causes of typhoid fever or paratyphoid fever, respectively. *Salmonella* can replicate both inside the vacuoles of host cells and in the external environment. *Salmonella* are the second most common pathogens isolated from humans with gastroenteric disease in developed countries (Buncic, 2006).

Salmonella Typhimurium and *S.* enteritidis occur in the gastro intestinal tract of animals, including livestock. The disease is self-limiting, but can be severe in young, elderly or otherwise IC (immunecompromised) people. *Salmonella* invade epithelial cells in the ileum and proliferate in the lamina propria and profuse, watery diarrhoea results. Some isolates produce a heat-labile enterotoxin, which initiates diarrhoea. Sequelae include post-enteritis reactive arthritis and reiter's syndrome and systemic infection can result. Individuals can develop carrier status of up to 6 months in duration. The infectious dose varies, from only a few CFU to >105 CFU, so growth of the pathogen in foods has not been a factor in all cases of foodborne salmonellosis, but appears to have been in some.

Foods known to have been vehicles of salmonellosis include poultry, eggs, and meat, milk, chocolate, coconut and frog legs. However, any faecally contaminated food can be implicated (Buncic, 2006). *Salmonella typhi* and *S. enterica* subsp. *paratyphi* cause the systemic diseases typhoid fever and paratyphoid fever, respectively. These pathogens occur in human faeces, and are spread via human faeces to the environment and to foods. Person-to-person transmission is common. The disease symptoms of typhoid and paratyphoid fevers are dissimilar to those of enteric salmonellosis (Buncic, 2006).

Salmonella penetrate the intestinal epithelium, possibly proliferating in macrophages and polymorphs, pass into mesenteric lymph nodes, liver or spleen then cause septicemia. Peritonitis and subsequent death can occur. Ulceration of the ileum can occur if organisms multiply in the bile of the gall bladder and cause re-infection. Any food could be a vehicle of infection if contaminated with human faeces. Foods known to have been vehicles of typhoid fever include raw milk, shellfish and meat. However, typhoid fever is predominantly spread by water contaminated with human faeces (Buncic, 2006).

2.2.11. Isolation and identification of Salmonella

Salmonella can be isolated either from tissues collected aseptically at necropsy or from faces, rectal swabs, environmental samples, food products and feedstuffs. When infection of the reproductive organs, abortion occurs, it is necessary to culture fetal stomach contents, placenta and vaginal swabs and, in the case of poultry, embryonated eggs. Individual samples for bacteriological tests should be collected as aseptically as possible by following the respective standards. Moreover, precaution should be taken to avoid cross contamination of samples during transit and at the laboratory. Packages should also be kept cool and accompanied by adequate information (OIE, 2005). Non-typhoidal *Salmonella* gastroenteritis is diagnosed when *Salmonella* is cultured from stool (Fuaci and Jameson, 2005). The isolation and identification of *Salmonella* can be performed using techniques recommended by International Organizations for Standardization (ISO-6579, 2002), and those recommended by the Global *Salmonella* Surveillance (GSS) and National Health Services for Wales (NHS) (Zelalem *et al*., 2011).

The two EU approved methods for *Salmonella* detection in food and animal feedstuffs are ISO-6579:2002 and NMKL 71 (Nordic Committee on Food Analysis) (Carrique-Mas and Davies, 2008). ISO-6579:2002 is sensitive, but complex and expensive. It consists of pre-enrichment of the sample in BPW followed by selective enrichment in MKTTn and RVS. From each enrichment medium, plating onto two agar media plates (one of which is Xylose-Lysine Desoxycholate [XLD] agar) is carried out after 24 h and 48 h of incubation. Up to five colonies per plate have to be confirmed, which may potentially involve the confirmation of up to 40 presumptive colonies (ISO-6579, 2002). Conventional cultural methods for the detection of foodborne *Salmonella* species generally consist of five distinct and successive steps. These are pre-enrichment in nonselective media and selective enrichment in broth media, plating on differential agar, biochemical screening and serological conformation (D'Aoust, 2001).

Pre-enrichment in non selective liquid medium

The *Salmonella* may be present in small numbers in environmental samples, faces from asymptomatic animals, animal feed and food and are often accompanied by considerably larger numbers of other *Enterobacteriace* or other families. There for it is necessary to use pre-enrichment media to assist the isolation. Furthermore, pre-enrichment is necessary to permit the detection of low numbers of *Salmonella* or injured *Salmonella* (ISO-6579, 2002).

Pre-enrichment with none selective broth medium for 18-24h at 35-37^{o}c allows the small numbers of *Salmonella*e, which may otherwise be killed by the toxic effect of enrichment media, to multiply, or it may help to resuscitate *Salmonella*e that have been sublethally damaged, e.g. by freezing, heating, exposure to biocides or desiccation. The use of short (6-8h) pre-enrichment for greater method brevity is not recommended because it fails to provide *Salmonella*e with sufficient time to adapt to its new environment, repair cellular damage and actively grow to high numbers (D'Aoust, 2001). Buffered peptone water is inoculated at ambient temperature with the test portion, and then incubated at 37 °C ± 1 °C for 18 h - 24 h. For large quantities, the buffered peptone water should be heated to 37 °C ± 1 °C before inoculation with the test portion (ISO-6579, 2002).

Enrichment in selective liquid medium

The recovery of *Salmonella* from food is often complicated by the presence of high numbers of indigenous micro flora in food that can interfere with the isolation of pathogens (Bakr *et al*., 2008). Success in isolating *Salmonella* is usually enhanced by the inoculation of incubated pre-enrichment broth into selective enrichment media (Read *et al*., 1994 and Davies, 1995). Selective enrichment media are used to select out the *Salmonella* population from all the organisms present in food, but no one is considered perfect. Three main families of selective enrichment media are recognized, selenite, tetrathionate (TT) and Rappaport-Vassiliadis (RV). Rappaport-Vassiliadis is currently recommended for *Salmonella* recovery from low and highly contaminated foods (Bakr *et al*., 2008). Rappaport-Vassiliadis medium with soya (RVS broth) and Muller-Kauffmann tetrathionate/novobiocin broth (MKTTn broth) is inoculated with the culture obtained in pre-enrichment. The RVS broth is incubated at 41.5 °C ± 1 °C for 24 h ± 3 h, and the MKTTn broth at 37 °C ± 1 °C for 24 h ± 3 h (ISO-6579, 2002).

The introduction of semi-solid selective enrichment media such as the modified semisolid Rappaport-Vassiliadis (MSRV) medium represented an improvement for fecal samples over previously used conventional selective enrichment broths, such as Rappaport-Vassiliadis (soya base) (RVS). There may be, however, differences in the performance of different brands of MSRV. Semi-solid selective enrichment media are easy to use, and negative results can often be obtained by direct visualization of the plates, leading to a more efficient isolation process (Read *et al*., 1994 and Davies, 1995).

Samples with a high level of competitive flora such as intestinal and other environmental samples may need to be incubated at higher temperatures (41.5°C), in order to provide *Salmonella* with an advantage over most competitive organisms. However, incubation at 35°C to 37°C may be more appropriate for detection of sensitive serovars such as *S.* Pullorum and *S.* Gallinarum in internal organs and tissues. Incubation above 43°C may be lethal for some *Salmonella* serovars (Busse, 1995) such as *S.* Dublin. Selective enrichment media are normally stored cold, so they should be allowed to warm up to at least room temperature before use to avoid reducing the temperature within the incubator, a potential problem where large numbers of plates are incubated together (Waltman, 2000).

Plating out and identification

From the cultures obtained in enrichment, two selective solid media are inoculated: xylose lysine deoxycholate agar (XLD agar); any other solid selective medium complementary to XLD agar and especially appropriate for the isolation of lactose-positive *Salmonella* and *Salmonella* Typhi and *Salmonella* Paratyphi strains; the laboratory may choose which medium to use. The XLD agar is incubated at 37 °C ± 1 °C and examined after 24 h ± 3 h. The second selective agar is incubated according to the manufacturer's recommendations. Brilliant green agar (BGA), bismuth sulfite agar and others could be used as the second plating-out medium (ISO-6579, 2002).

A simplified version of ISO-6579:2002 using one culture plate (Rambach) is currently used at the VLA for research purposes (Wales *et al*., 2006). Most of the recent studies confirm the good performance of the Rappaport- Vassilidis (RV) enrichment medium. These good results have been observed for various samples, as human feces, farm animals' and seabird feces (Monfort *et al*., 1994) and food products and reviewed by D'Aoust (1989). The authors have adopted this method based on a comparison of the performance of Rambach versus BGA and XLD on samples from the EU *Salmonella* baseline surveys for layers and broilers. A single Rambach plate performed as well as the combination of XLD and BGA, as long as pale orange colonies on Rambach were investigated (unpublished data). Confirmation of *Salmonella* with this method can be obtained within 72 h, although sometimes it has been possible to confirm and isolate within 24 h if incubation times are abbreviated and tests are carried out directly from selective media (Davies and Wray, 1994). The NMKL seventy one method (NMKL, 1999) was originally developed for the isolation of *Salmonella* in foods and feedstuffs. It is relatively simple and affordable, although it has shown limited sensitivity with veterinary faecal samples compared to other methods (Korsak *et al*., 2004). It includes a pre-enrichment stage in BPW followed by enrichment in RV and plating on to XLD plus a second medium of choice. Plating after 48 h of enrichment is optional, and may increase its sensitivity considerably, although in practice this is rarely done (Carrique-Mas and Davies, 2008).

Growth on selective enrichment media is plated after 24 h and 48 h onto solid plating media. Although a small number of additional isolates may occasionally be detected beyond 48 h, further plating is not considered cost-effective (D'Aoust *et al*., 1992). Non-

motile *Salmonella*e, including *S.* Pullorum and *S.* Gallinarum, and the *S.* Enteritidis vaccine strain present in Avipro *Salmonella* Vac E (Lohmann Animal Health) do not grow in MSRV, so they must be identified using different media. For *S.* Pullorum and *S.* Gallinarum the best results were obtained using direct enrichment in selenite cysteine and RVS (Proux *et al*., 2002).

Confirmation

For confirmation, it is recommended that at least five colonies be identified in the case of epidemiological studies. If on one dish there are fewer than five typical or suspect colonies, take for confirmation all the typical or suspect colonies. Streak the selected colonies onto the surface of pre-dried nutrient agar plates in a manner which will allow well-isolated colonies to develop. Incubate the inoculated plates at 37 °C ± 1 °C for 24 h ± 3 h and pure cultures is used for biochemical and serological confirmation (ISO-6579,2002).

Confirmation can be made using biochemically using triple sugar iron agar (TSI) (Oxoid CM0277, Basingstoke, England), Christensen's urea agar (Oxoid CM53, Basingstoke, England), lysine iron agar (LIA) (Oxoid CM381, Basingstoke, England), Voges Proskauer (VP), methyl red (MR) (Micromaster Thane, India), and Indole tests (Becton Dickinson, USA) (Zelalem *et al*., 2011). Typical *Salmonella* cultures show alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and (in about 90 % of the cases) formation of hydrogen sulfide (blackening of the agar) When lactose-positive *Salmonella* is isolated, the TSI slant is yellow. Thus, preliminary confirmation of *Salmonella* cultures shall not be based on the results of the TSI agar test only (ISO-6579, 2002).

Serological confirmation and serotyping- Agglutination tests, ELISA, anti-globulin and compliment fixation tests have been used to detect antibody responses to *Salmonella* infections (Quinn *et al*., 1999). The detection of the presence of *Salmonella* O-, Vi- and H-antigens is tested by slide agglutination with the appropriate sera, from pure colonies and after auto-agglutinable strains have been eliminated. This method of relies on the antibody/antigen reaction between a test culture and commercially prepared antiserum (ISO-6579, 2002).

Typing

Because of the importance of *Salmonella* in foodborne disease, numerous typing methodologies have been developed and have been used to trace salmonellosis outbreaks to the contaminated source and to delineate the epidemiology of *Salmonella* infections. Some of the typing techniques include serotyping and phage typing. These techniques are useful for defining relationships between strains (Botteldoorn *et al*., 2004).

Serotyping- Serotyping is based on the O and H antigens using slide agglutination test (Quinn *et al*., 1999). Most serotypes exhibit diphasic flagellar antigen expression by alternately expressing two genes, *fli*C (phase 1) and *flj* B (phase 2) which encode flagellins of different antigenicity. *Salmonella* serotyping methods recognize 63 district phase 1 flagellar antigenic factors and 37 phase 2 flagella antigenic factors although the latter are not always present (Mortimer *et al*., 2004).

Bacterial growth for serotyping should be taken from a triple sugar iron (TSI) agar slant or from nutrient agar as culture from selective media is often unsuitable for typing. A loopfull of culture of the *Salmonella* to be serotyped should be suspended in a drop of saline on a microscope slide and examined for autoagglutination. This this can occur with rough strains and will invalidate the serotyping. Smooth- rough dissociation occurs after subculture and most frequently from media containing carbohydrates. Smooth *Salmonella* to be serotyped is emulsified in a drop of 0.85% saline on a clean microscope slide. A drop of antiserum is added to and mixed well with the *Salmonella* suspension. The slide is rocked gently for about 30 seconds and the antige- antibody mixture examined for agglutination. The *Salmonella* is first tested againist antisera to the O (somatic) antigens and then the H (flagella) antigens (Quinn *et al*., 1999).

Phage typing- phage typing is based on the specificity of a given phage for its host bacterium, and this relationship allows one to use known phages to identify their specific hosts (Jay, 2000). Therefore, phage typing of *Salmonella* isolates is based on the sensitivity of a particular isolates to a series of bacteriophages at appropriate dilutions. This can be useful to determine whether isolates, which come for different places at different times, are similar or different in their reactions with specific sets of phages used for typing (Quinn *et al*., 1999).

2.2.12. Antimicrobial susceptibility tests and resistance profile

There are three test methods (disk diffusion, broth dilution and agar dilution). Antimicrobial susceptibility testing methods that consistently provide reproducible and repeatable results is when followed correctly (CLSI, 2008).

Disk diffusion - Disk diffusion refers to the diffusion of an antimicrobial agent of a specified concentration from disks, tablets or strips, into the solid culture medium that has been seeded with the selected inoculum isolated in a pure culture. Disk diffusion is based on the determination of an inhibition zone proportional to the bacterial susceptibility to the antimicrobial present in the disk. The diffusion of the antimicrobial agent into the seeded culture media results in a gradient of the antimicrobial. When the concentration of the antimicrobial becomes so diluted that it can no longer inhibit the growth of the test bacterium, the zone of inhibition is demarcated. The diameter of this zone of inhibition around the antimicrobial disk is related to minimum inhibitory concentration (MIC) for that particular bacterium/antimicrobial combination; the zone of inhibition correlates inversely with the MIC of the test bacterium. Generally, the larger the zone of inhibition, the lower the concentration of antimicrobial required to inhibit the growth of the organisms. However, this depends on the concentration of antibiotic in the disk and its infusibility (OIE, 2012).

Disk diffusion is straightforward to perform, reproducible, and does not require expensive equipment. Its main advantages are: low cost, ease in modifying test antimicrobial disks when required, can be used as a screening test against large numbers of isolates, can identify a subset of isolates for further testing by other methods, such as determination of MICs. Manual measurement of zones of inhibition may be time-consuming. Automated zone-reading devices are available that can be integrated with laboratory reporting and data-handling systems. The disks should be distributed evenly so that the zones of inhibition around antimicrobial discs in the disc diffusion test do not overlap to such a degree that the zone of inhibition cannot be determined. Generally this can be accomplished if the discs are no closer than 24 mm from centre to centre, though this is dependent on disk concentration and the ability of the antimicrobial to diffuse in agar (OIE, 2012).

Broth and agar dilution methods- The aim of the broth and agar dilution methods is to determine the lowest concentration of the assayed antimicrobial that inhibits the visible growth of the bacterium being tested (MIC, usually expressed in µg/ml or mg/litre). However, the MIC does not always represent an absolute value. The 'true' MIC is a point between the lowest test concentration that inhibits the growth of the bacterium and the next lower test concentration. Therefore, MIC determinations performed using a dilution series may be considered to have an inherent variation of one dilution. Antimicrobial ranges should encompass both the interpretive criteria (susceptible, intermediate and resistant) for a specific bacterium/antibiotic combination and appropriate quality control reference organisms. Antimicrobial susceptibility dilution methods appear to be more reproducible and quantitative than agar disk diffusion. However, antibiotics are usually tested in doubling dilutions, which can produce inexact MIC data. The selection of an AST methodology may be based on the following factors: ease of performance, flexibility, adaptability to automated or semi-automated systems, cost, reproducibility, reliability, accuracy, the organisms and the antimicrobials of interest in that particular OIE Member, availability of suitable validation data for the range of organisms to be susceptibility tested (OIE, 2012).

Salmonella species are leading causes of acute gastroenteritis in several countries and salmonellosis remains an important public health problem worldwide, particularly in the developing countries (Rotimi *et al*., 2008). The situation is more aggravated by the ever increasing rate of antimicrobial resistance strains (Zelalem *et al*., 2011). In recent years problems related to *Salmonella* have increased significantly, both in terms of the incidence and severity of cases of human Salmonellosis. Since the beginning of the 1990s, strains of *Salmonella* which are resistant to a range of antimicrobials including the first choice agents for treatment of humans have emerged and are threatening to become a serious public health problem. Drug resistant *Salmonella* emerge in response to antimicrobial usage in humans and in food animals so, selective pressure from the use of antimicrobials is a major driving force behind the emergence of resistance. Multi-drug resistance to critically important antimicrobials is compounding the problem (WHO, 2005). There are reports of high prevalence of resistance in *Salmonella* isolates from countries such as Taiwan (Lauderdale *et al.,* 2006), India (Mandal *et al.*, 2004, 2006), The Netherlands (Duijkeren *et al.,* 2003), resistant isolates from France (Weill *et al.*, 2006), Canada (Poppe *et al.,* 2006), and Ethiopia (Molla *et al.*, 2003).

A particular concern with *S.* Typhimurium DT 104 is that it has resistance to many antibiotics and often acquires resistance to others. Most strains are resistant to ampicillin, chloramphenicol, streptomycin, the sulphonamides and tetracycline. Recent resistance additions include resistance to trimethoprim and of particular concern, to the fluoroquinolones. Resistance to this latter group of antibiotics is a major worry as they are among the drugs of choice for the treatment of invasive *Salmonella* in humans. There is considerable debate as to what factors result in the emergence of antibiotic resistant strains of bacteria and it is alleged that antibiotic use in animals is part of the problem. Equally the use or misuse of antibiotics in humans for example also leads to the development of antibiotic resistance. The continuing development of antibiotic resistance may lead to sufficient pressure ultimately to restrict the antibiotics available to the veterinary profession for animal treatment (Gracey *et al*., 1999).

Antimicrobial resistant *Salmonella* are increasing due to the use of antimicrobial agents in food animals (Threlfall, 2002; Molla *et al*., 2003; Lynch *et al*., 2006; Molla *et al*., 2006; Zewdu and Cornelius, 2009) at sub-therapeutic level or prophylactic doses which may promote on-farm selection of antimicrobial resistant strains and markedly increase the human health risks associated with consumption of contaminated meat products (Molla *et al*., 2003; Molla *et al*., 2006; Zewdu and Cornelius, 2009). Cattle have been implicated as a source of human infection with antimicrobial resistant *Salmonella* through direct contact with livestock and through the isolation of antimicrobial resistant *Salmonella* from raw milk, cheddar cheese, and hamburger meat traced to dairy farms. Antimicrobial use in animal production systems has long been suspected to be a cause of the emergence and dissemination of antimicrobial resistant *Salmonella* (Alexander *et al*., 2009).

This spread of antimicrobial resistance through the food chain is regarded as a major public health issue (Threlfall, 2002 and Lynch *et al*., 2006). The appearance of both plasmid mediated antibiotic resistant against conventional anti- *Salmonella* drugs and chromosomal resistance to quinolones and fluoroquinolones has reduced therapeutic options for *Salmonella* septicemia in humans (Nor Elmadiena *et al*., 2012).

2.2.13. Treatment

Gastroenteritis caused by *Salmonella* is usually a self-limiting disease (Richards *et al*., 1993 and Fuaci and Jameson, 2005) and diarrhea resolves within three to seven days and fever within seventy two hours (Fuaci and Jameson, 2005). Accordingly therapy should be directed primarily to the replacement of fluid and electrolyte losses. Therefore, antimicrobials should not be used routinely to treat uncomplicated non-typhoidal *Salmonella* gastroenteritis or to reduce convalescent stool excretion (Richards *et al*., 1993). However, antimicrobial therapy should be considered for any systemic infection (Parry *et al*., 2002).

Antibiotic treatment usually is not recommended and in some studies has prolonged carriage of *Salmonella*. Neonates, the elderly, and the immunosuppressed (e.g., HIV-infected patients) with non-typhoidal *Salmonella* gastroenteritis are especially susceptible to dehydration and dissemination and may require hospitalization and antibiotic therapy (Fuaci and Jameson, 2005). Because of the increasing prevalence of antimicrobial resistance, empirical therapy for life threatening bacteremia or local infection suspected to be caused by non-typhoidal *Salmonella* should include a third generation cephalosporin and a quinolone until susceptibility patterns are known. Amoxicillin and trimethoprim-sulfamethoxazole are effective in eradication of long-term carriage. The high concentration of amoxicillin and quinolone in bile and the superior intracellular penetration of quinolone are theoretical advantages over trimethoprim-sulfamethoxazole (WHO, 2003a).

2.2.14. Economic and public health significance of Salmonella infections

Foodborne disease has emerged as an important and growing public health and economic problem in many countries during the last two decades. Frequent outbreaks caused by new pathogens, the use of antibiotics in animal husbandry and the transfer of antibiotic resistance to human are just a few examples (Rocourt *et al*., 2003). The pathogen of *Salmonella*, belonging to intestinal bacteria family, is one of the main pathogens causing food poisoning (Lianhua, *et al*., 2008). As the pathogen of foodborne infection, *Salmonella* is currently the leading pathogen of bacterial food poisoning in the world (Cheng, *et al*., 2008).

The incidence of non-typhoidal salmonellosis has doubled in the United States over the past two decades. Currently, the CDC estimates that there are 2 million cases annually, with 500 to 2000 deaths. Although more than 200 serovars of *Salmonella* are considered to be human pathogens, the majority of the reported cases in the United States are caused by *S.* Typhimurium or *S. enteritidis* (Fuaci and Jameson, 2005). Sheep and goats can be carriers of different *Salmonella* serovars, including *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium, the most important serovars for human infections (Schilling, 2012). Contacts with small ruminants pose a potential health risk to occupationally exposed subpopulations as well as the general public, but the risk depends strongly on the serotype involved (Hoelzer *et al*., 2011). The incidence of salmonellosis is highest during the rainy season in tropical climates and during the warmer months in temperate climates, coinciding with the peak in foodborne outbreaks (Fuaci and Jameson, 2005).

In most parts of the world, countries have seen dramatic and continuous increases in human outbreaks of salmonellosis, caused by infections in animals. In 2004, in the European Union (EU) alone, 192,703 human cases of salmonellosis were reported. These and similar data from other countries almost certainly underestimate the magnitude of the problem, as many cases of salmonellosis are not reported. The Centres for Disease Control estimate the annual number of non-typhoidal salmonellosis cases in the United States of America (USA) to be approximately 1.4 million (Forshell and Wierup, 2006).

In addition to human health implications, *Salmonella* is a pathogen of significant importance in worldwide animal production and the emergence of antibiotic-resistant strains, due principally to the therapeutic use of antimicrobials in animals, is a further threat to human and animal health (Forshell and Wierup, 2006). It also generates negative economic impacts due to surveillance investigation, and illness treatment and prevention (Gómez-Aldapa, *et al*., 2012). Financial costs are not only associated with investigation, treatment and prevention of human illness, fall in to the public and privet sectors and may be surprising, both in terms of the levels of costs incurred and the variety of affected. In the public sector, resources may be diverted from preventive activities in to the treatment of patients and investigation of the source of infection. In the private sector considerable financial burdens may be imposed on industry in general and on the food industry in particular, and last but not on the affected individual and his or her family (Sockett, 1991).

2.2.15. Prevention and control

In many urban centers, eating and drinking in public establishments, such as Hotels, Restaurants, and Snack bars is a common practice in many countries. These establishments prepare, handle, and serve large quantities of food and drink to large groups of people within a short period of time implying a possible risk of infections if sanitary and hygienic norms are not strictly followed. The world health status review indicates that the health problem of developing nations is mainly linked to inadequate sanitation (Kumie *et al*., 2002).

Better education of food industry workers in basic food safety and restaurant inspection procedures may prevent cross-contamination. Food handling errors can lead to outbreaks. Improvements in farm animal hygiene, in slaughter plant practices, and in vegetable and fruit harvesting and packing operations may help prevent salmonellosis caused by contaminated foods. Pasteurization of milk and treatment of municipal water supplies are highly effective prevention measures that have been in place for decades. Wider use of pasteurized egg in restaurants, hospitals, and nursing homes is an important prevention measure. In the future, irradiation or other treatments may greatly reduce contamination of raw meat (CDC, 2008). Strategies for reducing foodborne illness require a comprehensive farm-to-table approach, while *Salmonella* contamination from food handlers has been shown to make a significant contribution to human foodborne illness in several developing countries (Catherine *et al*., 2001).

Non-typhoidal *S. enterica* infections are a major public health problem world-wide and reduction of these diseases presents a serious and challenging problem. These diseases have several animal reservoirs. Large number of different *S.enterica* serovars cause gastroenteritis in humans probably makes vaccines very difficult to realize and/or use commercially. The incidence of non-typhoidal salmonellosis continues to rise along with rates of emergence of antibiotic resistant strains and increased centralization of food production. Thus, it is important to monitor every step of food production, from handling of raw products to preparation of finished foods. The prudent use of antimicrobial agents in both humans and animals is necessary to minimize the further emergence of antibiotic resistant strains (Getenet, 2008).

Furthermore, in order to control *Salmonella* infection, an individual should cook foods thoroughly, pasteurize milk and dairy products; avoid consumption of unpasteurized products, prevent cross-contamination of heat-treated foods, avoid undercooked or raw eggs, store heat-treated foods at less than 4°C or greater than 60°C to prevent growth, reduce carriage of livestock by vaccinating or dosing with antibiotics or probiotics, exclude infected or carrier-status individuals from handling food, control rodents and insects and dispose of sewage in a sanitary manner (Buncic, 2006).

2.2.16. Salmonellosis in Ethiopia

Even though *Salmonella* populations in different geographical areas or different hosts and environmental niche may undergo different evolutionary change, due to centralization of food production and distribution and population movement, *Salmonella* strains found in different countries of the world are believed to be clonally related (Winokur, 2001). *Salmonella* isolates in Ethiopia may have similar phenotypic and genotypic characteristics with isolates elsewhere in the world and non-typhoidal *Salmonella enterica* infection in children in Ethiopia is a major health problem and is caused by similar serovars to these reported from elsewhere in Africa: *S*. Typhimurium and *S*. Enteritidis (Getenet, 2008).

Salmonella infection most commonly occurs in countries with poor standards of hygiene in food preparation and handling and where sanitary disposal of sewage is lacking. It mainly occurs in the tropics and sub tropics in Africa, India, Pakistan South East Asia and South America (WHO; 1989; Lanata *et al*., 1990; Al-Lahham *et al*., 1990; Muleta and Ashenafi, 2001; WHO; 2003b; Senthikumar and Prabakaran, 2005).

Studies indicated the widespread occurrence and distribution of *Salmonella* in Ethiopia. In recent years the number of out breaks of *Salmonella* in humans has increased considerably in the country. Much more is known now about the extent of foodborne illness and how severe it can be, not just in terms of acute illness, but also in terms of long term consequences. Studies indicated various percentages of *Salmonella* isolates in towns of Ethiopia. Moreover, high percentages of *S. typhi* isolates have been found to be resistant for antimicrobial agents (Yismaw *et al*., 2007; Andargie *et al*., 2008; Abera *et al*., 2010). In addition, the very young, elderly and immunocompromized individuals are particularly

more susceptible to *Salmonella* infections at a lower infective dose than healthy adults. This is more important in developing countries such as Ethiopia where HIV/AIDS is highly prevalent and *Salmonella* is an important opportunistic infection in HIV/AIDS patients (Catherine *et al*., 2001).

In Ethiopia, minced beef is usually used for the preparation of a popular traditional Ethiopian dish known as locally "Kitfo" and most of the time it is consumed raw or medium cooked. The habit of raw meat consumption and the presence of *Salmonella* in minced beef indicate, in addition to the poor hygienic standards in food handling in the country, the presence of great public health hazards of *Salmonella* (Muleta and Ashenafi, 2001).

3. MATERIALS AND METHODS

3.1. Study site

This study was conducted between January, 2014 and May, 2014 at Dire Dawa Administration (DDA). DDA is located in the eastern part of Ethiopia and lies between 90^0 27' and 90^0 49'N latitudes and between 41^0 38' and 42^0 19'E longitudes, 515 Km from Addis Ababa, the capital city of Ethiopia. The total area of the administration is 128,802 hectare and the administration shares common boundaries with Somali National Regional States in the West, North and East and with the Oromia National Regional State in the South. Its altitude ranges from 960 meters above sea level (m.a.s.l) in the northeast to 2450 m.a.s.l in the South West. Using the 1500m contour as a line of separation, two agroclimatic Zones are known: the Kolla (below 1500m) and Woina Dega (above 1500m) (DDAEPA, 2011). The rainfall is bimodal and characterized by small rainy season from February to May and high rainy season from July to September. The dry season extends from October to January. The mean annual rainfall in the study area varies from 550mm in the lowland Northern part to 850mm in the Southern mountain ranges. The monthly mean maximum temperature ranges from 28.1°C, to 34.6°C. Likewise, the monthly mean minimum temperature varies from 14.5°C in December to 21.6°C in June (DDAC and Agricultural Bureau, 1998).

Dire Dawa city administration is a large town in Ethiopia and is situated along the Addis Ababa to Djibouti corridor, an asphalt road and railway that link the country to neighbouring Djibouti. The town hosts many truckers, long- and medium-distance intercity bus drivers, and cross-border businessmen. Dire Dawa has numerous economic and social institutions with a large number of permanent and temporary employees, including the Ethio-Djibouti railway station, textile and food factories, and higher-education institutions with a large number of students. Also there are many hotels and bars in the town (Amare, 2009).

3.2. Study population

The study population were all goat carcasses slaughtered in the abattoir and goat meat handlers (abattoir workers, butchers and consumers).

3.3. Sample size determination

For questionnaire survey and observation the sample size was determined purposively based on the willingness of the interviewees, ease for follow up and the chain of goat meat from abattoir to consumer. Accordingly, 85 participants consisting of 20 abattoir workers, 15 butchers and 50 consumers were included in the study.

For isolation and identification of *Salmonella,* the sample size was calculated based on 8.7% expected prevalence, 5% desired absolute precision and 95 % confidence interval using the formula recommended by Thrusfield (2005).

$$n = Z^2 p\,exp\,(1\text{-}pexp)\,/d^2,$$

Where n = =required sample size; pexp = expected prevalence and a desired absolute precision (d) of 0.05, Z = 1.96. Accordingly, the minimum sample size was calculated 122. To increases the precision of the estimate, the sample size was inflated and a total of 249 carcasses were considered.

3.4. Study design

A cross-sectional study involving microbiological analysis, questionnaire and observational survey was employed. The format of the questionnaire survey is presented in (Annex 8.3).

3.5. Sampling technique and sample collection

Carcasses samples were sampled by systematic random sampling technique. Swabs were taken according to the method described in ISO-17604 (2003). The abdomen (flank), thorax (lateral), crutch, breast (lateral), were the sampling sites. The sampling areas were delineated by using a (10 x 10 cm) aluminum foil templates. A sterile cotton tipped swab (2X3 cm) fitted with shaft, was first soaked in an approximately 10 ml of buffered peptone water (BPW) and rubbed over the delineated area horizontally and then vertically several times. Up on completion of the rubbing process, the swab was placed into the buffered peptone water used to wet the swab, breaking off the wooden shaft pressing against the inside of the universal bottle and disposed leaving the cotton swab in the universal bottle. Other swabs of the same types were used on the other marked areas and placed into the same container. A second dry sterile cotton swab of the same type was used as before over the entire sampled area as above and this swab was placed into the same container. Finally, by using ice boxes with ice packs the samples were transported to the College of Veterinary Medicine and Agriculture, Addis Ababa University.

3.6. Isolation and identification

Salmonella was isolated and identified according to the technique recommended by the international organization for standardization (ISO-6579, 2002) as shown in (Annex 8.1.). The bacteriological media were prepared according to manufacturer's recommendations (Annex 8.4).

3.6.1. Pre-enrichment and selective enrichment

The swab samples were pre-enriched in appropriate amount of buffered peptone water in (1: 9) ratio and incubated at 37°C for 24 hrs. Rappaport- Vassiliadis medium (RV) broth and Müller Kauffman Tetrathionate with novobiocin (MKTTn) broth were used for selective enrichment of the samples. About 0.1 ml of the pre-enriched sample was transferred into a tube containing 10 ml of Rappaport- Vassiliadis medium (RV broth) and incubated at 42 °C for 24 hours. Another 1ml of the pre-enriched broth was transferred into a tube containing 10ml of MKTTn broth and incubated at 37°C for 24 hours.

3.6.2. Plating out and identification

Xylose lysine desoxycholate (XLD) agar and brilliant green agar (BGA) plates were used for plating out and identification. A loop full of inoculums from each RV and MKTTn broth cultures were plated onto XLD and BGA plates and incubated at 37 ^{0}C for 24 hours. After incubation, the plates were examined for the presence of typical and suspect colonies. Typical colonies of *Salmonella* grown on XLD-agar have a black centre and a lightly transparent zone of reddish colour due to the colour change of the media (ISO 6579,2002) while H_2S negative variants grown on XLD agar are pink with a darker pink center. Lactose-positive *Salmonella* grown on XLD agar are yellow with or without blackening. Typical colonies of *Salmonella* on BGA are pink, 1 mm to 2 mm in diameter, and cause the color of medium to change to red. Five typical or suspected colonies were selected from the selective plating media, streaked onto the surface of pre-dried nutrient agar plates and incubated at 37^{o}C for 24hrs. Biochemical tests were done according to (ISO-6579, 2002) by using different biochemical tests that included TSI agar, L-lysine decarboxylation medium, urease and Indole production tests (Annex 8.6).

3.7. Antimicrobial susceptibility tests

The antimicrobial susceptibility testing of the isolates was performed by using the disc-diffusion method according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2002) and (CLSI, 2012). Four to five well-isolated colonies from nutrient agar plates were transferred into tubes containing 5 ml of Tryptone soya broth (Oxoid, England). The broth culture was incubated at 37ºC for 4 hours until it achieved the 0.5 McFarland turbidity standards. Sterile cotton swab was dipped into the suspension, rotated several times, pressing firmly on the inside wall of the tube above the fluid level to remove excess inoculums and swabbed uniformly over the surface of Muller Hinton agar plate (Oxiod, England). The plates were held at room temperature for 30 min to allow drying.

The susceptibilities of the isolates were tested for the following antibiotic discs: Ampicillin (AMP) 10 µg, Amoxicillin-clavulanic acid(AMC) (30 µg), Gentamicin (CN) 10 µg, Kanamycin (K)30 µg, Ciprofloxacin (CIP) 5 µg, Chloranphenicol (C) 30 µg, Trimethoprim (W) 2 µg , Sulphonamides (S3) 300µg, Tetracycline (TE) 30 µg, Nalodixic Acid (NA) 30 µg, Ceftriaxone (CRO) 30 µg, Streptomycine (S) 10 µg and Nitrofurans (F) 50 µg, were placed at least 15 mm apart and from the edge of the plates to prevent overlapping of the inhibition zones. The plates were incubated at 37ºC for 24 h. The diameter of the zones of inhibitions were compared with recorded diameters of the control organism *E. coli* ATCC 25922 and classified as resistant, intermediate, or susceptible according to the interpretive standards of the Clinical Laboratory Standards Institute (CLSI, 2012) (Annex 8.7).

3.8. Data management and analysis

The data collected from the questionnaire and observational survey and the results of the laboratory investigations were entered into Microsoft Excel and prepared for analysis. Descriptive statistics were performed using SPSS version 20 statistical.

4. RESULTS

4.1. Occurrence of *Salmonella* and antimicrobial susceptibility

Out of the total 249 carcass swab samples, 44 (17.7%) were positive to *Salmonella.* The highest level of resistance was observed for tetracycline (100%), nitrofurans (100%), streptomycine (81.8%) and kanamycin (79.5%). All isolates were susceptible to ciprofloxacin (Table 1). Of all the isolates, 43(97.7%) were multiple antimicrobial resistant (Table 2).

Table 1: Number of susceptible and resistant isolates by antimicrobials

Type of antimicrobial	**Number of isolates**		
	Resistant (%)	**Intermediate (%)**	**Susceptible (%)**
Ampicillin (AMP) 10 µg	24(54.5)	2(4.5)	18(40.9)
Amoxicillin-clavulanic acid (AMC) 30 µg	20(45.5)	14(31.8)	10(22.7)
Gentamicin (GEN) 10 µg	8(18.2)	12(27.3)	24(54.5)
Kanamycin (KAN)30 µg	35(79.5)	6(13.6)	3(6.8)
Ciprofloxacin (CIP) 5 µg ,	-	-	44 (100)
Chloranphenicol (C) 30 µg	20(45.5)	12(27.3)	12(27.3)
Trimethoprim (W) 2 µg	33(75)	1(2.3)	10(22.7)
Sulphonamides (S_3)300µg	19(43.2)	2(4.5)	23(52.3)
Tetracycline (TE) 30 µg	44(100)	-	-
Nalodixic Acid (NA) 30 µg	25(56.8)	12(27.3)	7(15.9)
Ceftriaxone (CRO) 30 µg,	10(22.7)	11(25)	23(52.3)
Streptomycine (S) 10 µg	36(81.8)	5(11.4)	3(6.8)
Nitrofurans (F) 50 µg,	44(100)	-	-

Table 2: Multiple antimicrobial resistances of the isolated *Salmonella*

Number	Antimicrobial resistance pattern (No.)	No. of isolates resistant (%)
Zero/one	None	0(0%)
Two	TET & NIT (1)	1(2.3%)
Four	STR, NAL, TET & NIT (1)	
	KAN,W,TET& NIT (2)	5(11.4%)
	S3, AMC.TET, NIT (1)	
Five	STR,KAN,NAL,TMP,AMP,TET& NIT (2)	7(16%)
	STR,KAN,NAL,TMP,AMC,TET& NIT (1)	
	KAN,S3,NAL,WAMP,TET& NIT (1)	
	STR,CAF,S3,NAL,TMP,TET& NIT (1)	
Six	STR,CAF,NAL,TET,GEN,& NIT (1)	2(4.5%)
	STR,S3,NAL,AMC,TET& NIT (1)	
Seven	STR,KAN,NAL,TMP,AMP,TET& NIT (2)	5(11.4%)
	STR,KAN,NAL,TMP,AMC,TET & NIT (1)	
	KAN,S3,NAL,TMP,AMP,TET& NIT (1)	
	STR,CAF,S3,NAL,TMP,TET& NIT (1)	
Eight	STR,CAF,KAN,NAL,TMP,TET,GEN& NIT (2)	10(22.7%)
	STR,KAN,NAL,TMP,AMC,AMP,TET&NIT(6)	
	STR,CAF,KAN,S3,CRO,TMP,TET& NIT (1)	
	STR,CAF,KAN,NAL,TMP,AMP,TET&NIT(1)	
Nine	STR,CAF,KAN,CRO,TMP,AMC,AMP,TET,& NIT (1)	6(13.6%)
	STR,CAF,KAN,NAL,TMP,AMC,AMP,TET& NIT (1)	
	STR,CAF,KAN,S3,NAL,TMP,AMC,AMP,TET& NIT (2)	
	STR,KAN,NAL,TMP,AMC,AMP,TET,GEN& NIT (1)	
	STR,CAF,KAN,CRO,NAL,TMP,AMP,TET& NIT (1)	
Ten	CAF,KAN,S3,CRO,NAL,TMP,AMC,AMP,TET & NIT (2)	4(9.1%)
	STR,CAF,KAN,S3,NAL,TMP,AMP,TET,GEN & NIT (1)	
	STR,CAF,KAN,S3,NAL,TMP,AMC,AMP,TET & NIT (1)	
Eleven	STR,CAF,KAN,S3,NAL,TMP,AMC,AMP,TET,GEN& NIT (2)	3(6.8%)
	STR,CAF,KAN,S3,CRO,NAL,TMP,AMC,AMP,TE & NIT (1)	
Twelve	STR,CAF,KAN,S3,CRO,NAL,TMP,AMC,AMP,TET& NIT(1)	1(2.3%)

Key: AMP = Ampicillin ; AMC = Amoxicillin-clavulanic acid; GEN = Gentamicin ; KAN = Kanamycin ; CIP = Ciprofloxacin; CAF = Chloranphenicol ;TMP = Trimethoprim ; S_3 = Sulphonamides ; TET = Tetracycline ; NAL = Nalodixic Acid ; CRO = Ceftriaxone ; NIT =Nitrofurans and STR = Streptomycine

4.2. Questionnaire and observational survey

4.2.1. Abattoir workers

Table 3 shows the knowledge, attitudes and practices of abattoir workers in relation to important parameters that potentially can influence the quality and safety of goat meat. All workers uses unclean knives while 18 (90%) of them keep equipments in unhygienic places.

Table 3: The knowledge, attitudes and practices of abattoir workers

Factors	Values	Frequency	Percentage (%)
Educational status	Illiterate	2	10
	Grade 1-8	10	50
	Grade 9-12	6	30
	Beyond grade 12	2	10
Placement in the abattoir	Slaughtering[a]	11	55
	Loading	4	20
	Washing stomach	3	15
	Washing the intestine	2	10
Job related training	Yes	6	30
	No	14	70
Job related medical test	Yes	8	40
	No	12	60
Know contamination as risk	Yes	16	80
	No	4	20
Clean clothing	Yes	2	10
	No	18	90
Hand washing	Before work	5	25
	After end of work	12	60
	Before and after work	3	15
Knives are clean	Yes	0	0
	No	20	100
Unhygienic equipments	Yes	18	90
placing	No	2	10

[a] =Cutting the throat, flaying eviscerating, splitting the carcass and carcass washing

Whilst eight of the respondents responded that unclean hand and equipments as major causes of carcass contamination, six considered falling on the ground as a major source of contamination. Washing the hands before and after work is practiced by only three of the interviewees and eighteen did not regularly put on clean protective clothing at work (Table 3). none of them responded that the faeces, skin and dirty water could possibly cause carcass contamination. Most (55%) interviewees consider that keeping hygiene is the role of the management while some (45%) of them think the role of management is setting standards for hygiene in abattoir and workers role is maintaining standards for hygiene in the slaughterhouse.

Direct observations revealed the absence of hot water, sterilizer and carcass retention room in the abattoir. During slaughtering equipments are placed on unclean surfaces. Knives were placed on the floor, in their (workers) mouth, on the skin of killed and in the anus of a slaughtered and hanged animals. The protective clothes were unclean, blood tinged and frequently in contact with carcasses. There were no separate compartments for final carcasses and animals to be slaughtered (Annex 8.6). The latrine is constructed far away from the abattoir and has no water, soap or other cleaning materials.

4.1.2. Butchers

Table 4 shows the knowledge, attitudes and practices of butchers in relation to important parameters that potentially can influence the quality and safety of goat meat. Among the fifteen butchers, thirteen acquired meat selling skills from observations and two of them from informal training. Fourteen of the butchers did not use protective clothes and twelve wash their hands with only water after work. All reported that they use a single knife for cutting meat and edible offal. Seven had worn jewelries and fourteen handled money while selling meat. All butchers cleaned their shop and equipments every day at end of the selling process by using water and clothes but one reported that uses soap in addition to water and clothes.

Table 4: The knowledge, attitudes and practices of abattoir workers

Factors	Values	Frequency	Percentage (%)
Educational status	Illiterate	2	13.3
	Grade 1-8	8	53.3
	Grade 9-12	4	26.7
	Beyond grade 12	1	6.7
Received job related training	Yes	2	13.3
	No	13	86.7
Apron (protective clothes)	Used	1	6.7
	Not used	14	93.3
Jewellery materials	Worn	7	46.7
	Not worn	8	53.3
Hand washing	Before work	-	-
	After work	12	80
	During work	-	-
	Not washed	3	20
Manner of washing hands	Rinsing with water only	12	80
	Using detergents and water	-	-
	Not wash	3	20
Handling money	Butcher with bare hand	14	93.3
	Cashier	1	6.7
Cleaning equipment's and shop	Every day at end of work(retailing) using water	15	100
Use disinfectants	Yes	1	6.7
	No	14	93.3
Cutting table	Single	14	93.3
	Separate for different organs and meat types	1	6.7

4.1.3. Consumers

The demographic characteristic of the 50 goat meat consumers is presented in Table 5 while the knowledge attitude and practice of the respondents is shown in table 6. Only 36% of them learned to high school and above while 30% of the respondents were uneducated and 62% of them were females (Table 5). Most of the respondents 28(56%) reported that they suffered food poisoning of which 22(78%) had medical attention and received antimicrobials and 2 (7%) were hospitalized. The symptoms of the cases were loss of appetite, diarrhea and weakness. Most 31(62%) reported that they prefer goat meat to other meat types. While 62%, 28% and 8% of them prefer to eat cooked, fried and raw meat respectively (Table 6).

Table 5: Demographic characteristic of the 50 goat meat consumers

Variable	Values	Frequency	Percentage (%)
Sex of respondents	Male	19	38
	Female	31	62
Age	9-28	21	42
	29-50	24	48
	51-90	5	10
Educational status	Illiterate	15	30
	Primary school	17	34
	Secondary school	10	20
	University	8	16

Table 6: Knowledge, attitude and practice of the consumers

Variable	Values	Frequency	Percentage (%)
Priority criterion to purchase goat meat	Freshness	16	32
	Low cost	5	10
	Low fat content	18	36
	Healthiness	5	10
	Mixed	6	12
How to use goat meat	Fried	14	28
	Cooked	31	62
	Raw	4	8
	All type	1	2
Consume raw goat meat	Yes	11	22
	No	39	78
Think cooked meat is always safe to eat	Yes	48	96
	No	2	4
History of food poisoning	Yes	28	56
	No	22	44
Meat slaughtered in abattoir is always safe to eat	Yes	45	90
	No	5	10
Have refrigerator	Yes	31	62
	No	19	38
Heard about *Salmonella*	Yes	13	26
	No	37	74
Know that meat can act as source of *Salmonella*	Yes	9	18
	No	41	82

5. DISCUSSIONS

The prevalence of *Salmonella*e in carcasses was estimated at 17.7%. In contrast to the previous studies in Ethiopia. For instance, Akafete and Haileleul, (2011) and Woldemariam *et al*., (2005) found that the prevalence of *Salmonella* from goat carcass swab was 8.3% at Modjo and 7.5% at Bishoftu, respectively. This difference could be due to differences in the hygienic and sanitary practices practiced in the abattoirs. Because, the current study concerns about the municipal abattoir, that has poor sanitation and hygienic standard in comparison to the export abattoirs. In addition to this workers in the current abattoir were found to be with poor general and personal hygiene and lack of knowledge in hygienic processing of meat, due to lack of training regarding hygienic and sanitation of slaughtering and working environment generally and there was no disinfectants, hot water and separate room for final carcass and live animals in the abattoir. The overall high level of carcass contamination with *Salmonella* is of special public health significance for a country like Ethiopia, where raw and under cooked meat is the favorite in most areas (Akafete and Haileleul, 2011).

Moreover, goats brought for slaughtering directly from market driving on foot, to be slaughtered without stay in lairage prior to slaughtering. But it is well recognized that, when animals are starved, *Salmonella* can survive and multiply in the rumen. Furthermore, healthy carriers intermittently excrete only a few *Salmonella*, unless they undergo some kind of stress such as transportation (Venter *et al*., 1994). Therefore, high contamination with *Salmonella* could be associated with high excretion of *Salmonella* with feces as source of contamination due to exposure to such predisposing factors as starvation, overcrowding in market and transportation.

The high level of carcass contamination with *Salmonella* is of special public health significance for a country like Ethiopia, where raw and under cooked meat is the favourite food in most areas (Akafete and Haileleul, 2011). In addition to eating raw and under cooked meat, most of the consumers does not have information about the risk of this contaminated meat, because they consider as it is safe to eat when slaughtered at abattoir therefore, consumers can also cross contaminate with other foods during processing. Furthermore, vegetable consumption at this study area is very common which is favourable for cross contamination with this pathogen during unhygienic preparation.

Resistance to multiple antimicrobials (97.7%) which was observed in current study was higher than other studies conducted in Ethiopia. For instance, Alemayehu *et al*., 2002; Endrias, 2004; Molla *et al*., 2004 and Zelalem *et al*., (2011)) reported 52%, 23.5%, 44.8% and 83.3%, respectively the multidrug resistance of *Salmonella* isolated from food of animal sources, animals and humans, as well higher than reports from elsewhere in the world (Stevens *et al*., 2006; Khaitsa *et al*., 2007; Al-Bahry *et al*., 2007; Elgroud *et al*., 2009; and Fadlalla *et al*., 2012), reported multidrug resistance of *Salmonella* isolates respectively as follow: 16%, 50% (from raw meats), (1.2%, 14.1% and 23.7%) *Salmonella* isolated from different type of samples, 51.7% and 37.82%. This difference could be because of that, antimicrobial-resistant *Salmonella* are increasing due to the use of antimicrobial agents in food animals at sub-therapeutic level or prophylactic doses which may promote on-farm selection of antimicrobial resistant strains and markedly increase the human health risks associated with consumption of contaminated meat products (Molla *et al*., 2003; Molla *et al*., 2006 and Zewdu and Cornelius, 2009).

Zewdu and Cornelius (2009) reported that the isolates of *Salmonella* from food items and workers from Addis Ababa were resistant to the commonly used antibiotics including streptomycin, ampicillin, and tetracycline. Furthermore, Zelalem *et al*., (2011) also indicated resistance of *Salmonella* isolates to commonly used antimicrobials including ampicillin, streptomycin, nitrofurantoine, kanamycine and tetracycline, with resistance rate of 100%, 66.7%, 58.3% and 33.3%, respectively. Similarly previous reports from South India (Suresh *et al*., 2006), from Nigeria (Akinyemia *et al*., 2005) and from Cameroon (Akoachere *et al*., 2009) indicated a similar 100%, over 90% and 100% respectively resistance to ampicillin. The result of the current research also indicated resistance of *Salmonella* isolates to commonly used antimicrobials including tetracycline, nitrofurans, streptomycine, kanamycin, and ampicillin with resistance rate of 100%, 100%, 81.8%, 79.5% and 54.5% respectively. However, higher resistance rate than previous reports with the exception of ampicillin and resistance to further drugs as well as to trimethoprim and nalodixic acid with resistance rate of 75 % and 56. 8% respectively was observed in this result. This difference could be due to the increasing rate of inappropriate utilization of antibiotics which favors selection pressure that increased the advantage of maintaining resistance genes in bacteria (McGeer, 1998 and Mathew *et al*., 2007). It is as well recognized that recent resistance additions include resistance to trimethoprim. The

continuing development of antibiotic resistance may lead to sufficient pressure ultimately to restrict the antibiotics available to the veterinary profession for animal treatment (Gracey *et al*., 1999). Moreover, this increase antibiotic resistance, in addition to public health problems, may lead to economic loss in the countries due to loss of exporting meat and animal products and cost of drug of choice to treat human and animals due to resistance development.

Ciprofloxacin showed a good antimicrobial activity against these *Salmonella* isolates. It was found that all of 44(100%) isolates were susceptible to ciprofloxacin. This result was comparable with previous reports by Molla *et al*., (2006) from central part of Ethiopia among isolates of sheep and goat meat, Akinyemia *et al*., (2005) from Nigeria, from human isolates and Zelalem *et al*., (2011), isolates of *Salmonella* from dairy farms in Addis Ababa. The effectiveness of such drugs like ciprofloxacin could be because of that they are not widely used in countries like Ethiopia and other African countries (Zelalem *et al*., 2011). In addition to this, effectiveness of this drug could be because of this drug is not well distributed to all societies and not simply prescribed rather than it is used as drug of choice in antibiotic resistant person. In addition to this, ciprofloxacin is not commonly used to treat animals in Ethiopia.

In the present study more than 60% of slaughter house workers and butchers had only a primary school education. Similarly more than 70% of slaughter house workers and butchers did not have job related training as regards to food hygiene but acquired their respective skills from observations. The results are in agreement with reports of Mekonnin *et al*. (2013) and Endale and Hailay (2013) who reported a primary school education and lack of job relating trainings in more than half of the slaughter house workers and butchers in Mekele city, Ethiopia. Therefore, these workers could cross contaminate and not handle meat hygienically due to lack of knowledge regarding hygiene, sanitation, risk of contamination and personal hygiene. However training of food handlers regarding the basic concepts and requirements of personal hygiene plays an integral part in ensuring safe products to the consumers (Adams and Moss, 1997) and food handlers should have the necessary knowledge and skills to enable them handle food hygienically (FAO, 1990).

The majority 8(40%) of the abattoir workers proposed unclean hand and equipment as the major causes of carcass contamination but none responded that the faces, skin and dirty water can cause carcass contamination. Besides, most consider that keeping hygiene is the role of the management while some of them think the role of management is setting standards for hygiene in abattoir and workers role is maintaining standards for hygiene in the slaughterhouse. It is well documented that, the fecal wastes from animal and humans are important source of bacterial contamination of the environment and foods chain (Ponce *et al*., 2006), and members of *Salmonella enterica* subspecies *enterica* are widely distributed in the environment and in thet56fcc intestinal tract of animals (Anjum *et al*., 2011). Thus, this research result indicates that most of the abattoir workers does not know source of meat contamination and their responsibility in hygienic management of goat meat accurately. Therefore, they can contaminate meat with such source of contamination unknowingly. The workers could not know how to minimize the risk of meat contamination if they do not know the source of meat contamination properly and their role in hygiene of the slaughter environments.

Good health is important for workers in the meat industry. Ill persons will often be carriers of more microorganisms (pathogenic microorganisms) than is usually the case. These microorganisms may then be transmitted to the meat/food with the risk of causing disease to the consumers. Illness must always be reported to the manager and/or the meat inspector of the slaughterhouse who will decide if the worker can stay or has to leave (Skaarup, 2011). Contradictory, this study result specify that among the respondents from abattoir house workers 60% of them reported that they never experienced job related medical test while 40% of them taken job related medical test once only in their work duration. In addition the respondents complained that even when they get ill the managers do not allow them to leave and to take rest and they complained that the managers were not professionals. The managers also indicated this problem occurrence is due to the demand of meat and the manpower in the slaughtering house for the area is not equivalent as a result every worker forced to work twenty seven days per month. This problem could be major source of meat contamination due to inappropriate processing of carcass and from ill persons working in the abattoir, which could be risk for the consumers.

Furthermore, every worker accountable in goat and sheep slaughtering activity allowed slaughtering about twenty to thirty five goat and sheep per day and every process from cutting the throat to final carcass preparation was covered by single person. As a result majority of the respondents complain that in the abattoir working quickly is preferred than slaughtering hygienically. Therefore, the workers worry is only to finish that all goats and sheep rather than slaughtering hygienically. This could be result in occurrence of high cross contamination of carcass which might be a risk for the consumers. This problems could be because of the managers are not professionals, to solve such problems they them self do not have the knowledge of food safety and hygiene.

The slaughtering process was unhygienic and unsanitary. There was no hot water, sterilizer and retention room and equipments rest on dirty surfaces. However, Akafete and Haileleul, (2011), reported that eviscerating knife significantly associated with carcass contamination and specific attention must be given to sterilization of knifes. Motsoela *et al*. (2002) also indicated that, it is salutary to note that knives must be immersed in water for two minutes at 82^0C to reduce the number of contaminating microorganisms. Contradictory to these facts, in current study site the same knife was used without sterilizing to slaughter different goats and sheep, for evisceration, cutting throat and skinning process. This could cause high carcass contamination with different foodborne pathogens unless it is solved.

Correspondingly, it was found that the equipment used for slaughtering process was rested on dirty surface during working, for instance they put their knife on ground, in their mouth own, on skin of other killed animal and in the anus of the hanged carcass and use it as it is, use the material they putted on the ground to collect water for washing carcass repeatedly, their protective clothes were full of blood and dirty and were in contact with carcass while they take the finalized carcass to the final rail and loading. In summery this type of area and slaughtering process can cause cross contamination of reedy to eat meat at different stage. In the same way D'Aoust, (1997) expressed that, all food that is produced or processed in a contaminated environment may become contaminated with *Salmonella* and be responsible for outbreaks or separate cases of disease as a result of faults in transport, storage, or preparation. Therefore the risk of carcass contamination might be increasing until it reaches the consumers at different stage due to above listed predisposing factors

such as in contact with dirt clothes wile loading, transportation, contaminated water in use of contaminated materials repeatedly and moving from one rail to another rail.

Removal of hides should be carried out in a manner that avoids a contact between the skin and the carcass and contact between the carcass and workers' hands, tools or equipment, which had previously contacted the hide. Knives and steels used in the de-hiding operation should be sterilised in water at 82°C (McEvoy *et al*., 200). In contrast to this information this research result indicates that, there was no separation between final carcasses and live sheep and goats those going to be killed. Consequently, there was high contact between skin of live sheep and goat with final carcass, since there was no separate room for final carcass and live animals. McEvoy *et al*., (200) expressed that, contamination can occur by direct contact between the hide and the carcass or by indirect transfer, i.e. from workers' hands, clothes, tools or factory equipment which has had previous contact with the hide.

During the life of the animal, the hide becomes contaminated with large numbers of microorganisms derived from a wide range of sources such as faeces, soil, water and vegetation, including pathogens such as *E. coli* O157:H7 and *Salmonella*. Many of these organisms are present on the hide of animals presented for slaughter. There is a positive relationship between the level of dirt on the hide and bacterial numbers on the carcass. The relationship is evident at sites on the carcass that are subjected to manual skinning during hide removal. Reduction of the bacterial loading on the hide of animals entering the slaughter process would limit the impact and scale of pathogen transfer from the hides to the carcass (McEvoy *et al*., 200).

The hygienic practices at the butcheries are unhygienic. Almost all butchers (93.3%) handle money with bare hands while processing meat and do not put appropriate protective clothes. Endale and Hailay (2013) reported that 91.7% of the butchers in Mekelle city handle money while processing the meat. In addition other study indicates that, Handling of foods with bare hands may also result in cross contamination, hence introduction of microbes on safe food. Because meat handlers are probable sources of contamination for microorganisms, it is important that all possible measures be taken to reduce or eliminate such contamination (Muinde and Kuria, 2005). As the paper money circulates among different individuals it could be contaminated with several pathogens including *Salmonella* and handling carcasses with bare hands that also handle such items may result in cross

contamination. In addition most butchers wash their hands after the selling process and use only water with no detergents and use single knife for edible offals and meat types and a single cutting board for all products without cleaning and sterilizing. The overall butchery practices are favorable for the contamination of goat meat.

Besides, all (100%) of the butchers reported that they clean their shop and equipments every day at the end of selling process using water and clothes, except single person who uses soap in addition to water and clothes. Contradictory, documented data indicates problems in cleaning with water and cloth alone as follows; blood proteins can create particular problems on porous surfaces, often giving rise to green/brown, and very resistant staining. Aged protein deposits can be quite hard, normally not scraping off easily with a fingernail. In addition to this, soil deposits in a food plant would be bad enough if problem was simply their rather unsightly appearance. But the fact that they harbor, nourish and protect spoilage or pathogenic microorganisms that are invisible to the naked eye makes the job somewhat harder. The soil must, of course, be removed as completely as possible by effective cleaning using the detergent. Water alone does not sufficiently wet to displace many types of soils or even to displace air from water- repellent or hydrophobic surfaces. In this case the water curls up under its own surfaces tension into droplets. Lack of wetting will prevent cleaning taking place. To achieve wetting of such surfaces, chemical agents who have particular surface properties are employed: 'surfactants' or 'wetting agents' (Gracey *et al*., 1999).

Of the total 50 goat meat consumers interviewed majority 31(62%) of the consumers reported that they prefer goat meat to other type of meat and 8% prefer eating raw to other types of preparations. But it is well recognized that, *Salmonella* infections are primarily of foodborne origin (Rounds *et al*., 2010) and can be transmitted by consuming undercooked meat (Fuaci and Jameson, 2005). Besides people can become infected following a failure of personal hygiene (Gracey *et al*., 1999). Although majority of them 62% of the respondent prefer to use cooked goat meat, this research outcome furthermore indicate that 30% and 34%, of the respondents were illiterate and learned to primary school only, who do not have adequate knowledge to prepare it hygienically. In addition 28% and 8% of them prefer to eat fried and raw goat meat which can be source of *Salmonella* to the consumers. Some facts indicate that *Salmonella* infections are primarily of foodborne origin (Rounds *et al*., 2010) and can be transmitted by means of using undercooked meat

(Fuaci and Jameson, 2005). Besides people can become infected following a failure of personal hygiene (Gracey *et al*., 1999). Moreover, during food handling and preparation pathogenic organisms may be transferred to food items by the handler both directly or by cross contamination through hands, surfaces, utensils and equipment that have been inadequately clean and disinfected between the preparation of different types of food (Linda du and Irma, 2005).

In addition, 90% of the consumers interviewed from the study area consider that meat slaughtered in abattoir is always safe to eat and prefers to buy observing the stump and 82% of then do not know that meat as source of *Salmonella*. However majority 28(56%) of the respondents reported that they usually suffered from food poisoning. This was witnessed from the laboratory result that out of the total 249 carcass swab sampled from apparently healthy slaughtered goat meat, it was found that 44 (17.7%) of carcass swab sample were positive to *Salmonella* on biochemical test which indicates the level of contamination with microorganism in the abattoir. Therefore, consumers of this contaminated meat could be predisposed to salmonellosis unknowingly without taking care during preparation and processing due to lack of information.

Respondents those reported history of food poisoning was specified that the symptom of their event were mostly loss of appetite, diarrhea and weakness. Correspondingly, Reda *et al*., (2011) reported that among 244 diarrheal stool samples collected from Hiwot Fana and Misrak Arbegnoch referral hospitals located in Harar which is closer to Dire Dawa, 28 (11.5%) of the case were found to be *Salmonella* which are resistant to commonly used antibiotics including ampicillin, amoxicillin and tetracycline. Therefore, symptom of their event could be associated with salmonellosis which might be transmitted to them from contaminated meat and other foods.

6. CONCLUSION AND RECOMMENDATIONS

The present study results revealed that high prevalence of *Salmonella*, presence of poor personal hygiene and sanitation, resistance of *Salmonella* to most antimicrobials except ciprofloxacin, low level of public awareness about contamination of goat meat with *Salmonella* and the associated probable risk in the study area. Consequently, goat meat provided to the consumers in the city was found to be poor quality and not safe for human consumption calling for urgent intervention. Based on the above conclusion the following recommendations are forwarded:

- Training programs must be provided on best practice of handling of meat for handlers and raising the level of awareness of people.
- It is better if other studies regarding bacterial load and sources of contamination in the abattoir and butcher shops performed.
- The manager of the abattoir should be professionals who well equipped with the concept of food quality and food safety
- The number of abattoir works should be proportional with number of goat slaughtered per day
- Since *Salmonella* is resistant to most common drugs, attention should be taken in selecting antimicrobials in treating *Salmonella* infection both in animals and human being based on antimicrobial susceptibility test
- Further study ought to be conducted to identify the source of contamination
- The degree of the risk of consumption of goat meat contaminated with *Salmonella* should be assessed
- The use of standardized procedures and applications like hazard analysis and critical control point in slaughtering and handling of goat meat in the abattoir should be applied

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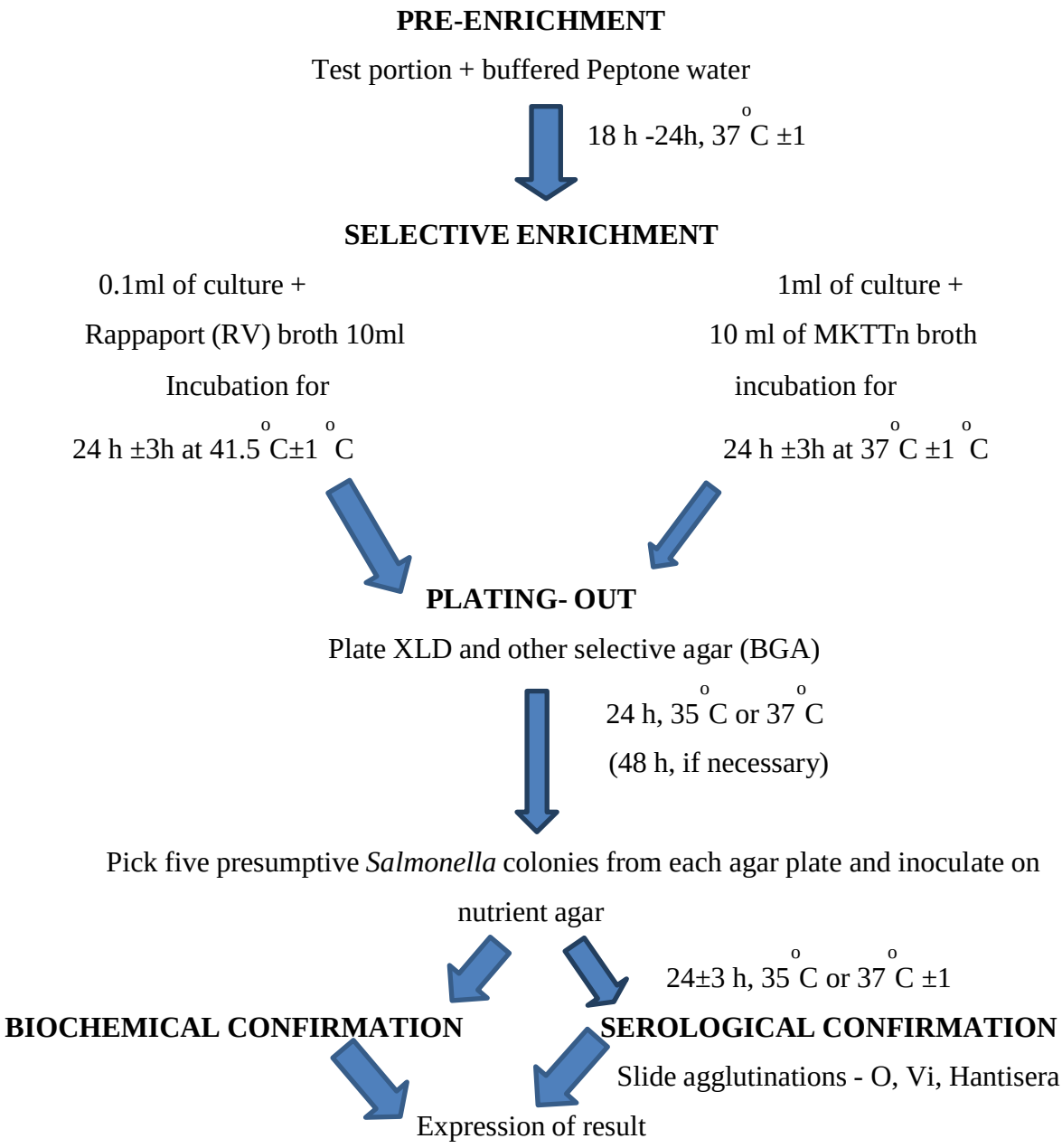
8. ANNEXES

Annex 8.1: Flow diagram showing ISO method for detection of *Salmonella*



Sources: ISO-6579, 2002**;** WHO, 2003c).

Annex 8.2. Plating and biochemical tests record sheet format used for *Salmonella* isolation

Sample ----------

Sample number	Date of sample collected	Colony characteristics on		Biochemical tests							Sample (+/-)
		XLD agar	BGA agar	Urease test	TSI test				L-lysine test	Indole	
1					Butt	slant	Gas	H_2S			
2											
3											
4											
5											
6											
7											
.											
.											
249											

Annex 8. 3: Questioner survey format and observed hygiene practice record for knowledge, attitude and practice analysis of different key informants along the meat pathway.

[

1. Abattoir workers

Date----------Questionnaire number----------

1. Name of slaughterhouse----------
2. Name of respondent ----------
3. Educational status: a) Illiterate b) Grade 1-5 c) Grade 6-8 d) Grade 9-12 e) Grade >12
4. Placement in slaughterhouses process: a) Stunner b) cutting the throat c) flayer

d) Eviscerator e) Splitter f) Carcass washer g) All h) other (specify)

A. Knowledge

A1. Do you play any other role in the slaughter process apart from the one mentioned above?

a) Yes [ ] b) No [ ]

A2. If yes, which one(s)? ---------- ----------

A3. If No, why not? ----------

A4. If your answer for no.4 is d, how frequently do you come across faulty eviscerations? -

A5. What do you do after faulty evisceration? ----------

A6. How do you handle goats presented for slaughter? _____

A7. Did you receive any job related training? a) Yes [ ] b) No [ ]

A8. If yes for A7; where were you trained? ----------

A8.1. If there was no formal training have you received informal training? ----------

A9. Have you undergone any job related medical tests to work in the abattoir ?

a) Yes [ ] b) No [ ]

A10. When was your last medical test done? a) one month b) two month c) three month d) six month e) one year

A11. What would cause carcass contamination?

1. Faeces
2. Dirty water
3. Handling with dirty equipment and hands
4. Other (specify)…….

A12.If carcass was contaminated by faeces, what would you do? (Open question)

1. Nothing
2. Wash the carcass
3. Call the meat inspector for advice
4. Other (specify)…….

A13. In your opinion, does contamination pose any health risk to meat consumers?

a) Yes b) No

A14. If No, why? ----------

A15. Propose way to end carcass contamination? ----------

Section B. Attitude

I will read you some statements about hygiene in the slaughter process. Please indicate whether you agree or disagree. Key: SA= strongly agree, A =agree, D=Disagree, SD=strongly disagree, and DK= don't know

No.	Question	SA	A	D	SD	DK
1	In this job, it is important to work quickly than keep the carcasses clean.					
2	People doing this job are more likely to get sick					
3	In this type of working environment keeping clean is easy					
4	A small amount of dirt on clothing or utensils will not cause any harm					
5	Health is more important than wealth					
6	Ensuring hygiene is mainly the role of management					
7	If meat is well-cooked then it is always safe to eat					

Section C. Practices (Butcher observation checklist)

Cuts/wounds covered with an appropriate waterproof dressing.	Yes----------No----------
Smoking or eating or chewing while working	Smoking----------chewing----
Clothes clean and completely free from any dirty or blood	Yes----------No----------
Hand washing before after and during cutting Meat	Before ----------After ------- During ---
How washed? Running water or bucket? Hot or cold? Brush or cloth? Soap?	Running water-------- bucket--------- Hot ------ cold---------Brush ------ cloth-----Soap----------
All knifes are completely clean and free from dirt and cracks and damages	Clean ----------undamaged -----
Knifes are cleaned before after and during Use	before ----------after---------- during use------
How often and when do you wash the equipment?	Every day at end of the process---------Once per weak--------once per month----------other(specify)-------
Is any disinfectant used? Write name of Disinfectant	Yes----------No----------
The source of water used in abattoir	Tap----------Well---------- Water vendor---------- other-------
Latrine available nearby	Yes----------No----------
Latrine has water soap paper towels for hand Washing	Water--------soap---------- paper--------towel---------- tissue paper----------
Equipments rested in dirty surface during Working	Yes----------No----------
Strict separation between clean and dirty Areas	Yes----------No----------
Veterinary inspectors present to examine the meat to be sold.	Yes----------No----------

Section D. perceptions

D1. What constraints do you experience in your work? ----------

D2. Do they affect your ability to achieve high levels of hygiene? ----------

D3. If Yes, in what way? ----------

D4. In your opinion, what role do you think the management should play in: ----------

a) Setting standards for hygiene in the slaughterhouse? ----------

b) Maintaining those standards? ----------

D5. In your opinion, what role do you think the workers should play in? ----------

a) Maintaining standards for hygiene in the slaughterhouse? ----------

b) Doing their work as much as possible quickly? ----------

2. Butcher shop workers (meat vendors).

Date completed: _____ Questionnaire number----------

1. Respondent Name: _____ Address: _____Occupation: _____
2. Name of butcher shops ----------
3. Educational status: a) Illiterate b) Grade 1-5 c) Grade 6-8 d) Grade 9-12 e) Grade >12
4. Did you receive any job related training? a) Yes [ ] b) No [ ]
5. If yes for 4; where were you trained?----------
6. If there was no formal training have you received informal training? ----------
7. How many carcass you receive per day? ----------
8. What is your selling capacity per day? _____
9. If the meat is not sold in a given day what will you do/ how do you handle? _____
10. How many knife you have and you use per day a) one b) two c) three d) four e) more (Specify)--------
11. How often and when do you wash the equipment? a) Every day at end of the selling b) Once per weak c) Once per month d) other (specify) -------
12. Who are most of your customers? _____
13. What would cause meat contamination? (Open question)
    a) Faeces

b) Dirty water

c) Handling with dirty equipment and hands

d) Other (specify)……

14. In your opinion, does contamination pose any health risk to meat consumers?

a) Yes [ ] b) No [ ]

15. If No, why? ----------

16. Propose way to meat contamination? ----------

B) Practices (Butcher observation checklist)

Cuts/wounds covered with an appropriate waterproof dressing.	Yes----------No----------
Smoking or eating or chewing while Working	Smoking----------chewing----
Apron (any protective clothes)	Yes----------No----------
Hand washing before after and during cutting meat	Before ---------After ------- During --- Not wash----------other-------
How washed? Running water or bucket? Hot or cold? Brush or cloth? Soap?	Running water-------- bucket--------- Hot ------ cold---------Brush ------ cloth-----Soap----------
All knifes are completely clean and free from dirt and cracks and damages	Clean ----------undamaged -----
Knifes are cleaned before after and during Use	before ----------after----------during use------
Is any disinfectant used? Write name of Disinfectant	Yes----------No----------
Wear Jewellery	Yes----------No----------
Handling money	Cashier ----------Butcher with bare hand
Cutting table	Single -------separate for different meats ----

Meat consumers

Date completed: _____Questionnaire number----------

17. Respondent Name: _____Sex: _____
    Age: _____Address: _____Occupation: _____
18. Educational status: a) Illiterate b) primary school c) high school d) university e) Master and PhD
19. From where you buy/ goat meat mostly? _____
20. What is your priority criterion to purchase meat?
    a) Freshness b) low cost(cheapness) c) low fat content d) healthiness
21. Which type of red meat you prefer? a) beef b) sheep c) goat d) camel e) all of them
22. How do you consume red meat? a) boiled b) fried c) cooked d) raw e) cooked in oven
23. Do you consume raw goat meat? a) Yes b) No
24. Do you think that cooked meat is always safe to eat? a) Yes b) No
25. How often do you consume meat? a) every day b) once in a week c) 1-3 times in a week d) 3-5 times in a week e) once per month f) most of the time
26. When do you consume meat most of the time? _____
27. History of food poisoning? a) Yes [ ] b) No [ ]
28. If yes symptoms? _____
29. How many times? a) once b) twice c) several times
30. If yes for no.7, what type of action taken? a) medical examination and antibiotic treatment b) other_____
31. Do you know any food poisoning/GIT disturbance associated with consuming of raw meat? _____
32. What are the symptoms? _____
33. Do you think that meat slaughtered in abattoir is always safe to eat? a) Yes b) No
34. How do you handle meat? _____
35. Do you have refrigerator? a) Yes b) No
36. Have you ever heard about *Salmonella* as foodborne disease? a) Yes b) No
37. Do you know that *Salmonella* can be transmitted through meat consumption a) Yes b) No

Annex 8.4: Composition and preparation of culture media and reagents

A) Buffered peptone water

Composition (g/Litre):

Enzymatic digest of casein	10.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate dodecahydrate (Na2HPO4·12H2O)	9.0 g
Potassium dihydrogen phosphate (KH2PO4)	1.5 g
Water	1 000 ml

Preparation: Add 15 gram of the components in the 1000 ml of distilled water, Mix well and distribute into universal bottle of suitable capacity to obtain the portions necessary for the test.

Sterilize by autoclaving for 15 min in the autoclave set at 121 °C.

B) Rappaport -Vassiliadis (RV) enrichment broth of 500 g (Oxoid, England)

Composition (g/Litre):

- Soya peptone …….5.0 g
- Sodium chloride……..8.0 g
- Potassium dihydrogen phosphate …..1.6 g
- Magnesium chloride …….40.0 g
- Malachite green…….0.04 g

Preparation: Weigh 30 g (the equivalent weight of dehydrated medium per Litre) and add to 1 Litre of distilled water. Heat gently until completely dissolved. Dispense 10 ml volumes into screw capped bottles or tubes and sterilize by autoclaving at 115 ^{o}C for 15 minutes.

C) Muller-Kauffman Tetrathionate (Novobiocine enrichment broth) (Oxoid Ltd., Basingstoke Hampshire , England)

Composition (g/Litre):

Tryptone 7.0; Soya peptone 2.3; Sodium Chloride 2.3; calcium carbonate 25.0; Sodium thiosulphate 40.7 and ox bile 4.75

Preparation: Suspend 89.5g in one litre of demineralized water, heat briefly to boiling. Do not autoclave! After cooling, add 20ml iodine potassium-iodide solition. Dispense evenly any precipitate. Potassium iodine solution: (5g KI, 4g I, PH 8.0 ±0.2 at 25^{o}c).

D) Xylose lysine deoxycholate agar (XLD agar) 500 g (Sifin, Berlin,Germany)

Composition (g/Litre):

Yeast extract…….. 3.0

- L-Lysine hydrochloride……..5.0
- Xylose…….3.75
- Lactose……....7.5
- Sucrose……7.5
- Sodium deoxycholate……..1.0
- Sodium chloride……..5.0
- Sodium thiosulphate……6.8
- Iron (III) ammonium citrate……0.8
- Phenol red……...0.08
- Agar……..16.5

Preparation: Suspend 56.68gm in 1000 (1 Litre) of distilled water by heating, with frequent agitation, until the medium starts to boil. Avoid overheating.

Adjust the pH, if necessary, so that after sterilization it is 7.4 at 25 °C.

Heat with frequent agitation until the medium boils and the agar dissolves. Do not overheat.

Transfer immediately to a water bath at 50 °C, agitate and pour into plates. Allow to solidify.

Immediately before use, dry the agar plates carefully (preferably with the lids off and the agar surface downwards) in the oven set between 37 °C and 55 °C until the surface of the agar is dry.

It is advisable not to prepare large volumes which will require prolonged heating.

E) BRILLIANT GREEN AGAR

Preparation: Suspend 29.0 g of the medium in one 500ml of distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 ibs pressure (121°C) for 15 minutes. Avoid overheating

F) Nutrient agar

Composition (g/Litre):

- Lab-Lemco powder……..1.0
- Yeast extract ……2.0
- Peptone…….5.0
- Sodium chloride……....5.0
- Agar……15.0

pH: 7.4 ± 0.2

Preparation: Dissolve 28g of the components or the dehydrated complete medium in 1000ml of distilled water, by heating if necessary. Sterilize for 15 min in the autoclave set at 121 °C. Transfer about 15 ml of the melted medium to sterile small Petri dishes and proceed.

G) Triple sugar/iron agar (TSI agar)

Composition (g/Litre):

'Lab-Lenco' powder 3.0; yeast extract 3.0; peptone 20.0; sodium chloride 5.0; lactose 10.0; sucrose 10.0; glucose 1.0; ferric citrate 0.3; sodium thiosulfate 0.3;Phenol red 9.5; agar 12.0. pH=7.4± 0.2 at 25^0C

Preparation: Suspend 65 grams in one Litre of distilled water and bring to the boil to dissolve completely. Sterilize in the autoclave set at 121 °C for 15 minutes. Dispense the medium into test tubes or dishes in quantities of 10 ml Allow to set in a slopped form to give a butt of depth 2. 5 cm to about 5 cm

H) L-Lysine Decarboxylation Broth of 500 g (Difco, Detroit, USA).

Composition (g/Litre):

- Bacto peptone……5 g
- Bacto yeast extract……3 g
- Bacto dextrose……...1 g
- L-lysine…….5 g
- Bacto Brom Cresol Purple… ……...0.02 g

Preparation: Suspend 14 grams in 1 litre distilled water or deionized water and boil to dissolve completely. Sterilize at 121 – 124 ^{o}C for 15 minutes. Final pH: 6.8 ± 0.2 at 25 ^{o}C.

I) Tryptophan Broth for Indole test

Composition (g/Litre):

Casein enzymic hydrolysate	10.0
Sodium chloride	5.0
DL- Tryptophan	1.0

Preparation: Dissolve 30gm of the components of tryptone broth in one litre distilled water.

Dispense 3 to 5 ml of the medium into each of tubes. Sterilize for 15 min in the autoclave set at 121 °C.

J) 0.5 McFarland standard

Composition: 1.17% $BaCl.2H_2O$ solution and 0.36N of 1% sulfuric acid (H_2SO_4).

Preparation: Add approximately 85Ml of 1% H_2SO_4 to a 100ml of volumetric flask, using a 0.5ml pipette add 0.5ml of 1.17% $BaCl.2H_2O$ dropwise to the H_2SO_4 while constantly swirling the flask. Bring to 100ml with 1% H_2SO_4.place a magnetic stirring in the flask and place on the magnetic stirrer for approximately three to five minutes. Examine solution visually to make certain it appears homogeneous and free of visible clumps. Dispense three to seven ml, cub tube tightly and seal with paraffin and keep at dark and room temperature.

K) Muller – hinton agar (**Oxoid, England**

Composition (g/Litre):

-Beef, dehydrated infusion from	300.0
-Casein hydrolysate	17.5
-Starch	1.5
-Agar	17.0

-pH 7.3 ± 0.1 at 25°C

Preparation: Add 38g to one litre of distilled water. Bring to the boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes.

L) Urea broth

Composition (g/l):

- Yeast extract ……0.1
- Potassium dihydrogen phosphate…….9.1
- Disodium hydrogen phosphate…….9.5
- Urea…….20.0
- Phenol red ……..…0.01

Preparation: 38.5 g/l was dissolved in Sterilize by filtration or dispense aliquots of approximately 3 ml into test tubes and sterilize for 5 minutes in a current of steam under mild conditions. Don't autoclaved. pH = 6.8.±0.1. The broth was clear and yellow-orange.

Annex 8.5. Pictures taken from the abattoir to demonstrate observational survey result

I. Equipment's rested in dirty surface during working and cloths full of dirty

(a= dirty cloths, b= knife rested on the ground)

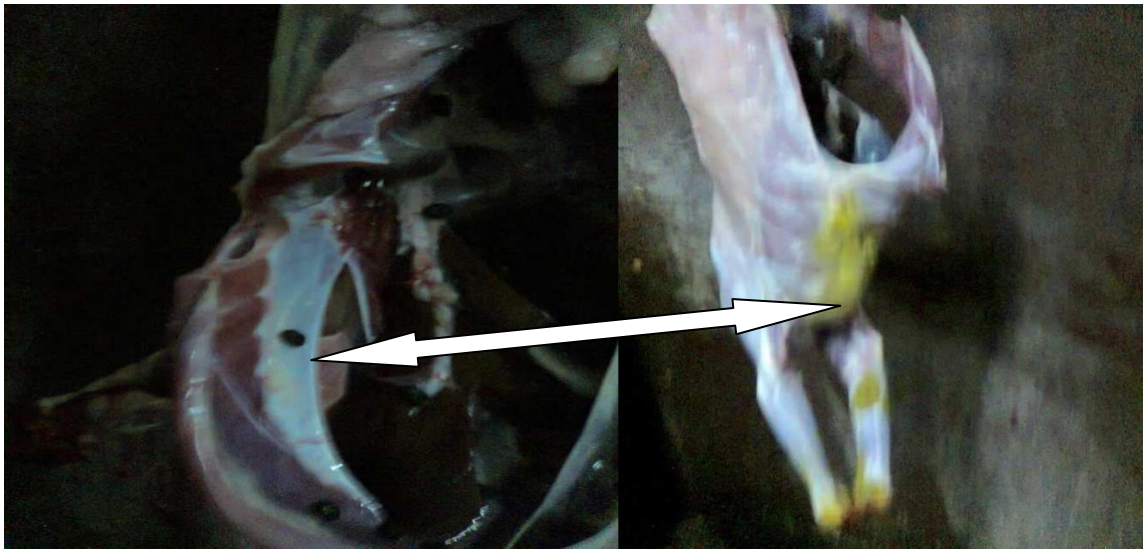
II. Equipment used for water collecting from another container and knife rested on the ground

III. Knife rested in dirty surface (a= in their mouth, b= on skin of other killed goat)

IV. (a= Smoking during work and personal unhygienic) and (b= kneel on the ground)

V. No separation of live sheep and goat carcass being dressed

(A= Final goat carcass, B= live sheep to be killed and C= carcass to be dressed) all in the same area.

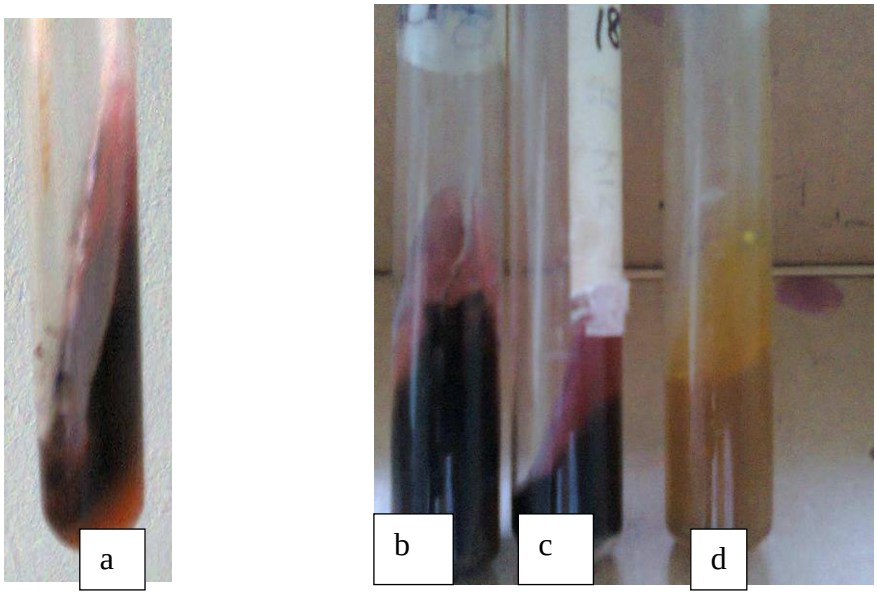


VI. **Contaminated carcass with cecal contents** (arrow indicates contaminated site with cecal content)

VII. No separation of skin and carcass (for Christian slaughter section), thus carcass and skin transported together

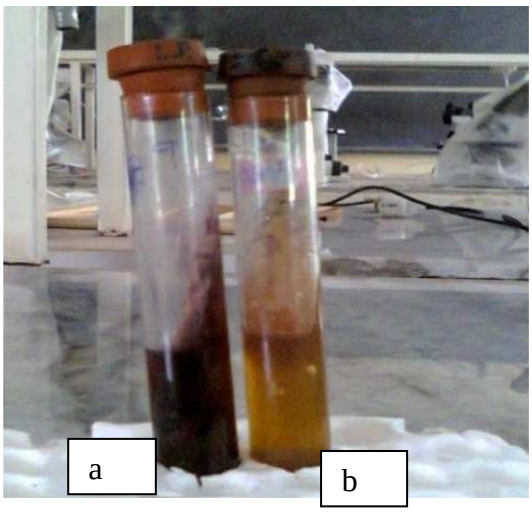
VIII. Close contact of workers cloth and final carcass during loading

Annex 8. 6. Pictures shows biochemical and drug sensitivity tests



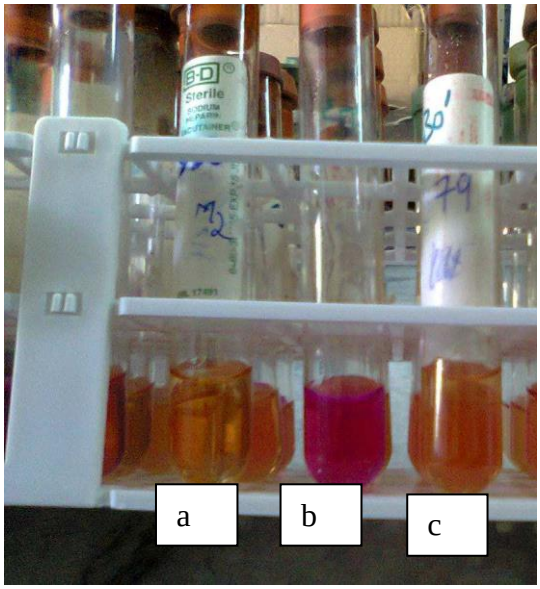
I. Triple sugar/iron agar (TSI agar)

(a=Lactose and/or Sucrose fermentation negative, H_2S production and glucose fermentation Posative), (b= Lactose and/or Sucrose fermentation negative, H_2S production positive), (c= Lactose and/or Sucrose fermentation negative, H_2S production and gas production positive) and (d=uninoculated)

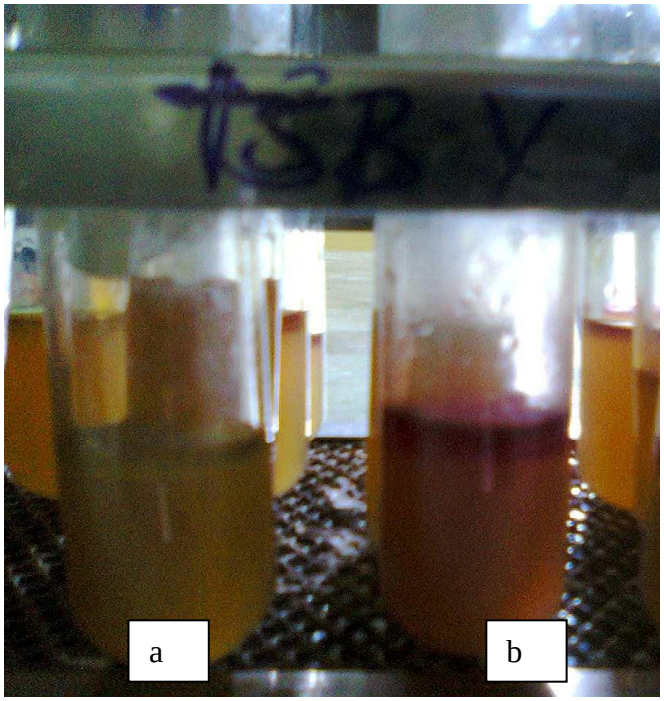


II. L-Lysine decarboxylation medium

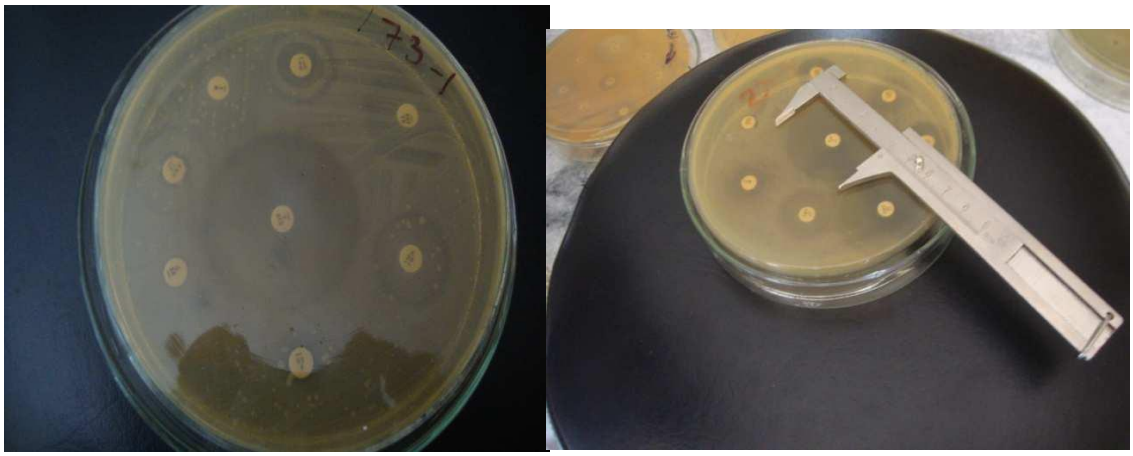
a= Lysine decarboxylation and H2S production positive and b=Lysine decarboxylation and H2S production negative



III. Urea broth (a= uninoculated (control), b= Urease positive and c= urease negative)



IV. Indole test (a= Indole negative a= Indole positive



V. Antimicrobial susceptibility test

Annex 8. 7: Performance standards for antimicrobial susceptibility testing of *Salmonella*

No	Antimicrobial Agent	Disc Code	Potency	Resistant	Intermediate	Susceptible
1	Ampicillin	AMP	10 µg	≤13	14-16	≥17
2	Amoxicillin-clavulanic acid	AMC	30 µg	≤13	14-17	≥18
3	Ceftriaxone	CRO	30 µg	≤19	20-22	≥23
4	Chloranphenicol	C	30 µg	≤12	13-17	≥18
5	Ciprofloxacin	CIP	5 µg	≤20	21-30	≥31
6	Gentamicin	CN	10 µg	≤12	13-14	≥15
7	Kanamycin	K	30 µg	≤13	14-17	≥18
8	Nalodixic Acid	NA	30µg	≤13	14-18	≥19
9	Streptomycine	S	10µg	≤11	12-14	≥15
10	Tetracycline	TE	30 µg	≤11	12-14	≥15
11	Trimethoprim	W	5µg	≤10	11-15	≥16
12	Sulphonamides	S3	300µg	≤12	13-16	≥17
13	Nitrofurans	F	50 µg	≤14	15-16	≥17

Source: CLSI, (2012).